Volume LXXII Number 5

THE BOTANICAL GAZETTE

Editor: JOHN M. COULTER

NOVEMBER 1921

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EDITED BY

JOHN M. COULTER

With the assistance of other members of the botanical staff of the University of Chicago

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VOLUME LXXII NUMBER 5

THE
BOTANICAL GAZETTE

NOVEMBER 1921

DECAY OF BRAZIL NUTS

EDWIN ROLLIN SPENCER

(WITH PLATES VIII–XII AND THREE FIGURES)

Introduction

Brazil nuts, Para nuts, Cream nuts, etc., are the seeds of *Bertholletia nobilis* Miers and *B. excelsa* Humb. and Bonpl. The nuts are harvested in the months of January, February, and March, when the heavy pericarps containing the seeds fall to the ground. They are collected and transported from the forests to the seaports at a time of year when heat and moisture favor fungous growth, and often a cargo reaches New York 30 per cent spoiled. The United States Bureau of Chemistry holds that nuts are adulterated food if more than 15 per cent are spoiled, and requires that such nuts be shelled before being placed on the market. In spite of these measures, however, Brazil nuts reach the consumer containing from 10 to 25 per cent of spoiled nuts. There were 43,076,348 pounds of Brazil and Cream nuts shipped into the United States in 1919 (7). It is probable that half of this amount, or 21,538,174 pounds, were retailed in the shell. It is conservative to estimate the loss through spoiled nuts at 10 per cent of this amount, or 2,153,817 pounds, an approximate money loss, at 40 cents per pound, of $861,526.80, which falls directly upon the consumer. Brazil nuts do not become rancid very readily, and for this reason they are not placed in cold storage during warm weather as are most other nuts. They are heaped in piles in supposedly dry and often very

hot rooms where, when moisture is present, fungous growth is favored.

There is a wide difference in shell porosity of Brazil nuts, and a positive correlation between fungous infection and shell porosity has been demonstrated. Two two-pound samples of Brazil nuts purchased at two different grocery stores were tested for porosity of shell as follows. The nuts were taken one at a time and dipped first in 95 per cent alcohol to prevent the collection of surface bubbles, and then plunged two or more inches beneath the surface

TABLE I

Percentage spoiled	Very porous		Slightly porous		Least porous		Spoiled in sample
	Cracked	Spoiled	Cracked	Spoiled	Cracked	Spoiled	
	Sample I						
100	4	4	.....	.....	.....	.....	.....
34	.....	.....	26	9	.....	.....	.....
12	.....	.....	.....	.....	49	6	.....
25, in sample	.....	.....	.....	.....	.....	.....	19
	Sample II						
50	2	1	.....	.....	.....	.....	.....
38	.....	.....	21	8	.....	.....	.....
7	.....	.....	.....	.....	70	5	.....
15, in sample	.....	.....	.....	.....	.....	.....	14

of hot water contained in a tall beaker. The heat-expanded air arose in bubbles from the pores of the shell. Table I shows the results obtained. The conclusion to be drawn from these data is that the most porous nuts are not necessarily spoiled, but readily become infected when conditions favor infection, while the least porous nuts are much less subject to infection. It is quite possible that so long as the water content of the nut is sufficient to support fungous growth, nuts with very porous shells may be entered and spoiled if storage temperature is favorable. Such infections probably account for the high percentage of spoiled Brazil nuts bought of retailers whose wholesale patrons have scrupulously complied with the ruling of the Bureau of Chemistry when the nuts were purchased at port.

Although the use of nuts as foods and confections has recently become extended and general, there is but little concerning nut diseases in the literature, and studies of the diseases affecting the nuts only for the most part have been superficial. MANGIN (17) described a "black rot" of chestnuts caused by *Harziella castanae* Bain., and found it to cause a 26 per cent loss of nuts gathered late in the season. VON IVANOFF (31), in studying *Trichothecium roseum* Link, found it in pure state on the kernels of *Corylus avellana* and *Pinus cembra*, and this, with RAND'S (23) work on *Coniothyrium caryogenium* Rand, is the only serious investigation of nut parasites that has been made. MARTZ (18) reports a species of *Cephalothecium* on pecans in Florida. KUHL (13) isolated *Aspergillus flavus* Mont. from Brazil nuts, but his description of both disease and fungus is meager.

A few parasites of nut plants cause diseases of the nuts themselves. The most serious disease of this kind is that produced by *Pseudomonas juglandis* Pierce, which, according to SMITH (27), attacks the nuts as well as other growing parts, and "a nut in such cases is deformed in shape . . . . and the kernel . . . . is only poorly developed." PIERCE (21) says that in young nuts the kernel is destroyed. Chestnuts are affected by *Endothia parasitica* (Murr.) A. and A. RUMBOLD (24) says that the hyphae of this parasite spread throughout the kernel. The kernel spot of pecan produced by *Coniothyrium caryogenium* Rand has incidently been studied by TURNER (30), and by RAND (23). In addition to these studies, there have been some reports on storage results (6, 29), and MCMURRAN (16) mentions what he considers a non-parasitic disease, the "black-pit" of pecan.

The aim of the present investigation was to isolate and identify as many as possible of the more important fungi and bacteria causing deterioration of Brazil nuts. Seven distinct organisms have been isolated, studied, and their etiological relation to the nut deterioration demonstrated. The remainder of the paper comprises the methods of study and descriptions of the organisms isolated.

Methods

The nuts studied were obtained from two wholesale firms in Chicago and from retail grocery stores in Champaign and Urbana,

Illinois, and were purchased at different times during the year 1919–20. Each nut was superficially examined and the shell carefully removed by cracking it with a hammer. The diseased nuts were dropped, shell and all, into suitable sterilized glass dishes, one nut in each dish. A number was assigned to each, but only those diseases which were most prevalent and which presented the most conspicuous diagnostic features were selected for study.

A preliminary examination was made of thin razor sections of diseased tissue mounted in water or xylol, in order to discover whether fungi or bacteria were present, and if so, to ascertain their general relation to the host tissues. If this examination showed any single species of organism to be predominant, isolations were made either by direct transfer to cornmeal agar plates, or by dilution plating as the case required. These isolations were from both exterior and interior portions of the nut, and when from the interior were carried out in the following manner. The nut meat was cut into with a flamed scalpel and carefully broken apart. A central portion of about 4 sq. mm. area was carefully removed with a flamed scalpel from one of the newly exposed surfaces, and discarded. In the center of the cavity thus made small pieces of diseased tissue were loosened with the point of the scalpel, and immediately carried in a sterilized loop to the surface of cornmeal agar plates.

Following isolation, the next step was to determine whether the fungi isolated were responsible for the various diseased conditions. Two methods were used; first, pieces of mycelium or a few spores were placed on sterile kernels contained in sterilized one-inch test tubes; and second, pieces of mycelium or a few spores were placed upon strips of sterile nut meat, 50 $\mu \times 5 \times 10$ mm., which were contained in tubes of sterile water, one strip on the side of the tube just above the surface of the water and the other in the water. By the first of these methods the rotting power of the parasite was made evident within a few weeks by the softening of the whole mass. With the second method results were obtained more quickly by more or less complete dissolution of the very thin sections employed. The following media were used in the case of every organism.

CORNMEAL AGAR.—This was prepared as recommended by SHEAR (**26**), except that the medium ready for filtration was poured into precipitation cones and autoclaved. After solidifying, the precipitated dirt at the apex of the inverted cone was removed and the clean agar melted and tubed.

BRAZIL NUT AGAR.—Fifty grams of Brazil nut kernels which were free of, or easily freed from, their inner seed coats were ground in a nut grinder (a Russwin no. 1 Food Cutter was used), and steeped for one hour at from 58° to 60° C. in 1000 cc. of distilled water, counterpoised, and filtered. Fifteen grams of powdered or crude agar was added and the mixture boiled for ten minutes, counterpoised, poured into precipitation cones, and autoclaved at 15 pounds for fifteen minutes. After solidification the agar was removed from the glass cone and placed on a clean sheet of paper. After removing the sediment the dirt-free agar was returned to the precipitation cone and again autoclaved. The resolidified agar cone was in two distinct layers, and the translucent layer was the one used.

NUT PLUGS.—It was found possible, by flaming a scalpel after each stroke, to cut out nut plugs of considerable size which were free from contamination. The kernels from which such plugs were to be cut were placed in a 2 to 1000 solution of mercuric chlorid where they remained for thirty minutes. They were taken from the solution one at a time, held by one end between thumb and finger, and shaped by cutting away a thin layer with a sharp scalpel, flamed after each stroke. When the plug was finished it was cut off after placing it within the mouth of the reclining one-inch test tube. Nut plugs made in this way remained uncontaminated for several months.

NUT STRIPS.—Strips of nut meat, 50 $\mu \times 5 \times 10$ mm., were cut on a microtome and preserved in absolute alcohol. When used they were taken from the alcohol with sterilized forceps and placed in sterile water in a Petri dish. From this they were removed with a sterilized loop and placed in test tubes containing sterile water, as already described.

AUTOCLAVED RICE.—This medium was made by placing two or three grams of rice and twice the volume of water in test tubes and autoclaving.

MICROTOME SECTIONS.—These were made in order to show the morbid histology in comparison with the normal histology. Because of the oil content of the nut, ether was found to be the best killing and fixing agent. The ether was replaced after three days with chloroform, and the ordinary schedule for imbedding with this reagent followed (**2**). After sectioning it was found that the oil content had not been sufficiently lessened, and that without its removal a distinct view of the structures could not be secured. To obviate the difficulty the sections were fixed to the slide, treated with xylol, xylol and absolute alochol, absolute alcohol, and then flooded four or five minutes with ether. The slide was held horizontally between thumb and finger, and the dissolved fats collected on the under side of the slide, from which they were wiped off before placing the slide in 95 per cent alcohol. The slide was kept in each of the 95, 85, and 70 per cent alcohols for about five minutes, and then flooded with Pianese IIIb for 15 minutes (**32**), washed with distilled water, 70, 85, and acidulated 95 per cent alcohol, 95, 100 per cent alcohol, 100 per cent alcohol and xylol, xylol, and mounted in balsam. The stain shows the host tissue in red and the fungus in green.

Two of the fungi produced pycnidia which were sectioned for study. These were taken from cultures on cornmeal agar, killed and fixed with chromacetic fixing fluid, and stained with Bismark brown, following the usual schedule for this stain. The pycnidia were removed from the culture on a strip of agar, usually about $1 \times 2 \times 4$ mm. in size, which remained intact throughout the process and served well in the orientation of the specimens in the imbedding dish.

FREEHAND SECTIONS.—It was occasionally found necessary to stain razor sections made for the preliminary study of the diseased tissue. The sections were cut as thin as possible and placed in a watch glass contained in a Petri dish. The watch glass was then filled with ether and the Petri dish closed. When the ether had evaporated, 95 per cent alcohol was poured over the sections and allowed to stand for ten or fifteen minutes. This was followed with 85, 70, and 50 per cent alcohols for five minutes each. The sections were then transferred to slides prepared with albumen

fixative, flooded with water, and allowed to stand over night. They were stained with Pianese IIIb, or with jod grün-erythrosin in the following manner. They were placed in 95 per cent alcohol for five minutes, flooded with jod grün (1 per cent solution in 95 per cent alcohol) for thirty minutes, washed with 95 per cent alcohol, then absolute alcohol, flooded with 1 per cent solution of erythrosin in clove oil for forty-five minutes, washed with absolute alcohol, cleared with carbol-turpentine clearer, and finally washed in xylol and mounted in balsam. This proved to be the most satisfactory of any method tried for staining mycelium in the tissue.

PROTEOLYTIC ENZYMES.—The Brazil nut agar serves well to show the presence or absence of certain extracellular proteolytic enzymes. The proteid precipitates to which the opacity of the agar is due are digested by the enzymes, and a transparent halo, which enlarges as the thallus or colony enlarges, surrounds the growth. All the organisms studied were tested for the presence of these enzymes. The enzymes were precipitated from cornmeal broth in which an *Actinomyces* or a *Bacillus*, the two most active enzyme producers, was grown. The broth was poured into Piorkowski culture flasks to a depth of 0.25 inch, about 200 cc. being required for each flask, and inoculated. After ten days the culture was filtered through paper and enough 95 per cent alcohol added to the filtrate to make 80 per cent alcohol. Three days later a fluffy white precipitate had collected at the bottom of the precipitation cones, and the excess alcohol was siphoned off (**12**). From 25 to 50 cc. of absolute alcohol was added to the precipitate and immediately filtered. Before the precipitate had become dry it was again washed with 50 cc. of absolute alcohol, and while still moist was removed to a desiccator containing sulphuric acid, and allowed to remain there for two days. The hard, gray material was then scraped from the paper to be redissolved in sterile water when used.

MORPHOLOGY OF BRAZIL NUT.—The kernel of the Brazil nut, as it is ordinarily removed on cracking the shell, is covered with a thin, dry coat which may be quite loose or may adhere very tenaciously. The embryo within, principally radicle, is completely

enveloped with a layer of endosperm 40 to 50 μ in thickness, and as reported by YOUNG (**35**) is "plainly differentiated into cortical and medullary tissues separated by a layer of procambium along which rudimentary vascular bundles are arranged at intervals." There is a single, somewhat irregular layer of epidermal cells just beneath the endosperm, and within 5 mm. of the distal end are the two very minute, unequal cotyledons which "measure about 750 by 175 μ" (**1, 25**). The cortical and medullary cells are similar in shape and size, and are largely filled with oil and proteid bodies. The endosperm cells, arranged with the long diameter at right angles to a median plane, are especially rich in proteids (**28**). The procambium cells with the long diameter at right angles to that of the endosperm cells contain few or no proteid grains (fig. 1).

The outer seed coat or shell is made up of two layers; the outer with its crinkled surface is light brown and softer in texture than the inner layer, which is dark brown and has a glazed inner surface. In the angles of the shell this layer seems to be of two layers which divide, leaving spaces filled with still another tissue that is lighter in color and softer in texture than the outer of the shell layers. In the micropylar angles of the seed is a narrow cavity. Such cavities are termed by BERG (1) the "loculi spurii in testa," and extend the entire length of the shell. This open channel probably serves as the usual entrance of the parasites of the nut (fig. 1).

The tissues of the seed, taken in order, beginning with the shell are: (1) outer seed coat in two distinct layers, with a softer tissue filling the triangularly prismatic corners; (2) the thin inner seed coat which may or may not adhere to the kernel; (3) the endosperm layer, two cells thick; (4) epidermis, a single-celled layer; (5) cortex, of large storage cells; (6) procambium layer, generally three cells thick; (7) the medullary tissue, of large storage cells.

Diseases of the Brazil nut

1. BLACK CRUST

GENERAL DESCRIPTION.—Fully 5 per cent of all diseased Brazil nuts are affected with black crust, but there is no external indication of their condition, since the shells are normal in color and the

weight is the same as that of sound nuts. When the shell is removed the kernel presents a dull black appearance which, if the whole nut is affected, reminds one of a large sclerotium. A cross-section of the diseased kernel shows the blackened portion to consist of a thin layer, 100–250 μ in thickness, apparently having no connection with the tissues beneath, which, aside from their light brown color and their pronounced nut odor, appear to be normal

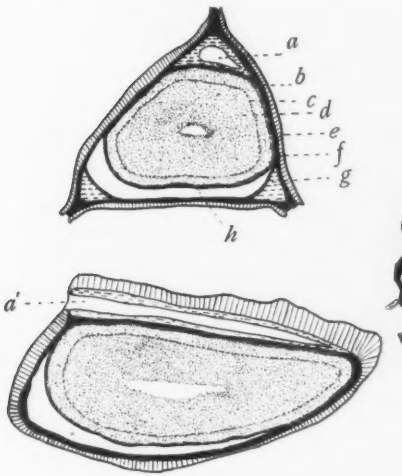

FIG. 1

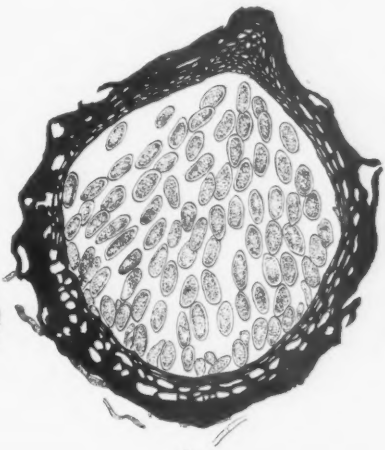

FIG. 2

FIGS. 1, 2.—Fig. 1, Diagrammatic drawings of cross and longitudinal sections of Brazil nut: *a* and a^1, locule in testa; *b*, endosperm layer; *c*, procambium layer; *d*, medullary tissue; *e*, outer layer of outer seed coat; *f*, inner layer of outer seed coat; *g*, soft tissue filling corners of shell; *h*, inner seed coat; fig. 2, pycnidium with immature spores, *Pellioniella macrospora* n. sp.; ×500.

(figs. 34–36). The diseased nut meats are frequently found covered with various fungi, chiefly *Penicillium* or *Aspergillus*, with black crust under the mold. A study of microtome sections shows that the mycelium is in the endosperm layer, the affected cells of which are hypertrophied (figs. 34, 35). The cortical cells of the radicle immediately beneath are not parasitized, but their contents are markedly changed. The proteid grains are almost or quite lacking in the epidermal and outer layers of the normal cortex, while in diseased nuts there is a superabundance of small proteid grains in

this region (figs. 34, 35). As it is possible to find nuts seemingly free from any other organism, the black crust fungus is easily isolated. Small pieces of tissue taken aseptically from immediately below the crust on direct plating gave pure cultures.

MORPHOLOGY.—The mycelium on cornmeal agar is of two kinds, that made up of cells which are longer than wide, and that with cells either nearly globular or wider than they are long (figs. 18, 19). The long-celled type predominates, both in the aerial and the submerged mycelium, except near pycnidia, where the shorter cells are most in evidence. The long cells are 14–32 μ in length by 3.5–14 μ in width, the short ones measuring 10–18 μ in diameter. Both types are thick-walled and black when mature, and both have granular contents (figs. 19, 20). In autoclaved rice and in the black crust of diseased nuts the hyphal cell is transformed until the hyphae suggest chains of conidia (figs. 14, 20). These cells are black, 10–15×5–8 μ in size, and contain one or two guttulae. They readily break away from the hyphae and function as spores.

Pycnidia are produced sparingly, and only along the border of a thallus where it comes in contact with another thallus, either of the same or of some other species. No pycnidia were found on diseased nuts or on any of the cultures except those on cornmeal agar plates. They are black, smooth, globose-conical, beaked, and 150–350 μ in diameter. The beak is 100–250 μ in length (figs. 2, 21).

The spores are borne at the base of the pycnidial cavity on short, hyaline, often septate conidiophores which are interspersed with narrow strap-shaped, hyaline, continuous paraphyses that are from one to six times as long as the conidiophores, the conidiophores being 5–14 μ in length by 3–5 μ in width (fig. 13). The spores are at first hyaline, unicellular, 26–36×14–20 μ in size and irregular in shape, but with maturity they become sooty black, striated, uniseptate, regular in shape and uniform in size, being 28×14 μ (fig. 15).

CULTURE CHARACTERS.—On cornmeal agar plates the fungus grows at the rate of 0.5–0.7 mm. per day at room temperature. The thallus is at first milk white, and the margin of it remains uncolored so long as it is increasing in size. After five or six days

the central portion becomes green, and a few days later turns sooty black. The thallus then is made up of three concentric rings, the outer white, the next green, and the innermost black (fig. 11). As the thallus ages it shows marked zonation, and becomes entirely black when growth ceases. Aerial mycelium is produced on all parts of the thallus, but is most luxuriant in the central area. On Brazil nut agar the growth is very much slower than on cornmeal agar, usually 0.3 mm. per day at room temperature, and the entire thallus remains hyaline. On nut plugs the growth was very weak, but a crust similar to that of naturally diseased nuts was formed after three months. On autoclaved rice the growth was vigorous, and several characteristic color reactions were noted (fig. 8). Eight days after inoculation: aerial mycelium snow white with line of Antique Green[1] below; rice grains in contact with glass, white bordered with Cerulian Blue; all interstices with greenish tints. After fifteen days: aerial mycelium white with lower border line Prussian Blue, almost black; contacts of grains with glass white bordered with Prussian Blue; interstices purple tinged.

The hyaline immature spores as well as the black mature ones germinated readily. The immature spores occasionally germinated in ten minutes after planting, while more than an hour is required for the germination of the mature spores, but the germ tubes of the mature ones soon outstrip those of the immature (figs. 16, 17). There is no change in either spore, except a slight swelling in germination, the immature spore remaining unicellular. This phenomenon of the germination of immature as well as mature spores has been pictured by HIGGINS (**11**) for a related species.

TAXONOMY.—The morphological characters of the fungus are those of *Pellioniella* Sacc., but according to SACCARDO (**25**) there is but one species in the genus, *P. deformans* Penz. and Sacc., whose spore measurements are a little more than half those of the Brazil nut parasite. The fungus, therefore, is given the name *Pellioniella macrospora*.

[1] The nomenclature used in describing colors throughout these investigations is that given in ROBERT RIDGEWAY'S Color standards and color nomenclature, published by the author, Washington, D.C. 1912.

Pellioniella macrospora, n. sp.—Pycnidia sparse, smooth, carbonaceous, globose-conical, beaked, 150–350 μ in diameter, beak 100–250 μ in length. Conidiophores at base of pycnidial cavity, hyaline, often septate, 5–15×3–5 μ. Paraphyses hyaline, strap-shaped, continuous, 5–50 μ. Immature conidia hyaline, unicellular, irregular, 26–36×14–20 μ; mature conidia sooty black, striated, uniseptate, regular, 28×14 μ.

HABITAT.—Parasitic on endosperm of seed of *Bertholletia nobilis* Miers and *B. excelsa* Humb. and Bonpl.

2. WHITE MOLD

GENERAL DESCRIPTION.—White mold is not so common as black crust, and is probably responsible for less than 1 per cent of the Brazil nut decay, but it is a real factor in this loss. The diseased nut is normal in external appearance, but is below normal in weight. When cracked, the white, fluffy mycelium is seen to cover the entire kernel, but soon after exposure the hyphae collapse and the yellowed endosperm becomes visible through the mycelial mass. A pronounced musty odor arises from the newly shelled nut, but the taste of the diseased meat has nothing to distinguish it. A cross-section of the nut kernel shows three typical features of the disease: (1) the white moldy covering; (2) the endosperm layer, sulphur-yellow in color and more than twice as thick as in the normal nut; and (3) irregular cracks and cavities in the radicle, all filled with white mycelium and spores. The mycelium penetrates to the center of the radicle. The hyphae in the tissue are very tenuous, less than 2 μ in diameter, and are usually so closely associated with the cell walls of the host tissue as to make a study of them *in situ* very difficult, but the cell walls of the diseased nut are penetrated by them. The fungus was isolated as described, but spore dilutions made by touching a sterile loop to the mycelial mass in the internal check of the kernel gave pure cultures also.

MORPHOLOGY.—Mycelium taken from the nut, from nut plugs, and from other media was uniform in character. The following description is of mycelium taken from cornmeal agar plates. The cells measure 20–70×3.5–10.5 μ, and are hyaline with granular contents and guttulae. Anastomosis of cells is of frequent occur-

rence, especially in the submerged hyphae, while in the aerial mycelium simple loops and coils are common (fig. 24). The hyphae are unconstricted at the septa, and unbranched cells are of quite uniform size throughout their length. Cells bearing branches are swollen at the points from which the branches arise.

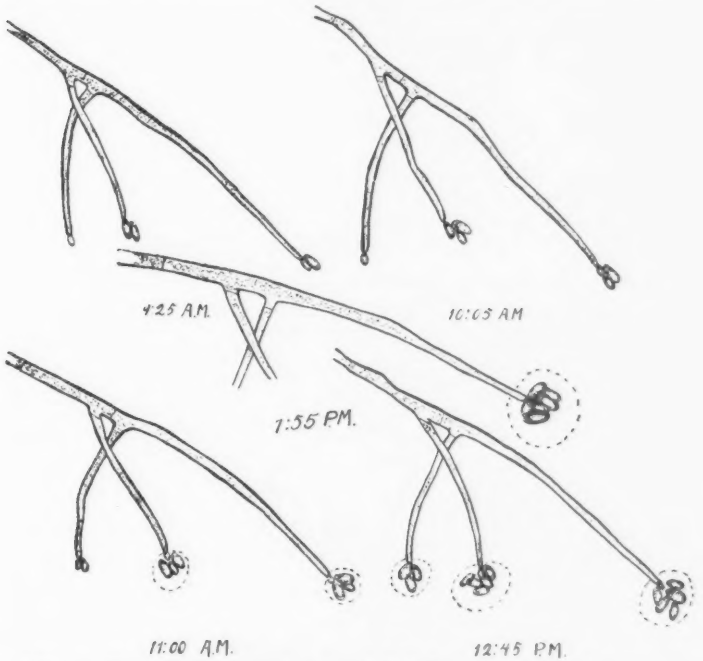

FIG. 3.—Development of spore mass of *Cephalosporium bertholletianum* n. sp.; dotted line shows size of water drop surrounding mass; ×100.

The conidiophores, 50–90 μ in length, are in most instances simple branches from either a principal filament or from its branches. They are spindle-like, with rounded tips from which the spores are cut off (fig. 3). The single-celled, oblong-elliptical, hyaline conidia are 8–12×3.5–5.4 μ in size, and contain two guttulae (fig. 23). They collect, as they are produced, in a spherical mass at the end of the conidiophore. A drop of water surrounds the spore mass after the third spore arrives (fig. 3).

CULTURE CHARACTERS.—On cornmeal agar the fungus grows rather rapidly, 0.5–1.0 cm. daily at room temperature. The thallus is very regular and is distinctly zonated after reaching a diameter of 4 or 5 cm. There is a dense growth of white aerial mycelium on the older portions of the thallus. On Brazil nut agar the characters are as previously stated, except that the growth is a little more rapid, and a halo 3 mm. wide, due to the digestion of the solid proteids, surrounds the thallus. On nut plugs the fungus grows luxuriantly and destroys the nut meat without giving off any appreciable odor. A large amount of fluffy mycelium is the characteristic feature of its growth on nut plugs as it is on the nut in the shell. On autoclaved rice the growth is vigorous and a pink tinge appears in the medium after two days. After ten days four colors are distinguished; where the rice is in contact with the glass in the older portions, Ochraceous Buff, in the younger, Venetian Pink; interstices between the grains are filled with mycelium through which a Jasper Pink color shows, in the older portions; in the younger portions it is Light Hortense Violet.

The nut strip above the water is soon covered with the dense mycelium, and is appreciably shrunken within five days. The strip in the water remains intact, but the water is soon filled with the mycelium which makes its way upward from the strip at the bottom. In hanging drop the spores germinate with a single germ tube, which in the most vigorous, at room temperature, may attain the length of the spore in two hours after planting. Spore production in hanging drop at room temperature proceeds at the rate of about one spore per hour per conidiophore. The conidiophore lengthens and increases in diameter as the conidia are cut off at the end. The process is very like spore production of *Trichothecium* as described by LINDAU (**14**).

TAXONOMY.—The fungus evidently belongs to *Cephalosporium*, but none of the species of this genus as reported by SACCARDO (**25**) has characters sufficiently like the Brazil nut parasite to permit it to be classified as one of them. *C. fructigenum* McAlp. (**15**) has spores of almost identical shape, size, and appearance, but it has knobbed conidiophores and oblong spore masses which are not present in this species, which therefore is described as new.

Cephalosporium bertholletianum, n. sp.—Conidiophores hyaline, simple or dichotomously branched, 50–90 μ long, 2- to 4-septate; spore mass globular; conidia hyaline, unicellular, oblong-elliptical, guttulate, 6–12×3.5–5 μ, ends obtuse.

HABITAT.—On radicle of seed of *Bertholletia nobilis* Miers and *B. excelsa* Humb. and Bonpl., causing decay.

3. DRY ROT

GENERAL DESCRIPTION.—The shell of the nut affected by dry rot is mottled, but of somewhat lighter shade in its darkest areas than the shells of normal nuts, and the weight is much below normal. The cracked shell appears to be filled with a kernel which adheres more closely to the shell than is usual, but which is so similar in color and general appearance to that of sound nut kernels that it might easily pass casual observation as such, although in reality it is merely a mass of mycelium. Small pieces of mycelium taken from this mass swell to approximately twice their size when placed in water. Under the microscope the hyphae were seen to be irregularly branched and septate. No conidiophores were seen, but what appeared to be unicellular elongated conidia of greatly varying length were occasionally found.

MORPHOLOGY.—The hyphae which make up both the aerial and the submerged mycelium are irregularly branched, and more or less constricted at the septa. The cells are 14–90 μ long by 3.5–11 μ wide, hyaline with granular contents and guttulae (fig. 44). Anastomosis frequently occurs, especially in older thalli, when spores falling on the medium germinate, producing a tube which unites with the cell of an older hypha, another germ tube, or another spore (figs. 29, 48).

The simple conidiophores are borne at any place along the hyphal strand, seldom more than two being produced by a single cell. Branched conidiophores are rare. Thalli resulting from direct planting of mycelium taken from the diseased nut produce but few conidiophores, and rarely more than single-celled conidia. Conidia from transferred cultures are from 1- to 8-celled, subcylindrical, slightly sickle-shaped, without pedicel, and conical at base (fig. 42).

No perithecia were found, but sclerotia were formed on autoclaved rice. These are dark gray and 500–1000 μ in diameter. Terminal chlamydospores are produced on 60-day old cultures. They are globular or oblong, with an average mean diameter of 14 μ, and with a scarcely perceptible yellow tinge (fig. 45).

CULTURE CHARACTERS.—Pure cultures are easily obtained by directly planting pieces of mycelium taken from the innermost portion of the mycelial mass. On cornmeal agar the rate of growth averages 0.5 cm. daily at room temperature. The thallus is arachnoid and regularly zonated, the zones averaging 0.5 cm. in width. Aerial mycelium covers the entire thallus but is most luxuriant in the central area, and there it is tufted. On Brazil nut agar the growth is more dense and a little more rapid than on cornmeal agar, its rate being from 0.7 to 1.0 μ at room temperature. An extra-cellular, proteolytic enzyme is secreted, causing a halo of 3–5 mm. in width in the medium surrounding the thallus. The aerial mycelium is more luxuriant on this agar than on the other.

The fungus grows vigorously on nut plugs, so that in a few days the plugs are enveloped with the snow-white mycelium, while a putrid odor is exhaled. After two or three months the plugs are completely reduced and only a mycelial mass remains. There is no color change on autoclaved rice until it shrinks away from the tube, when it is Maize Yellow. The fluffy, white, aerial mycelium surmounts the rice column and covers its sides as the growth proceeds downward. Apparently complete destruction of the rice is accomplished within two months. On carrot plugs the growth is not so rapid as on other media, but is marked by an abundance of white aerial mycelium. It is without color change. On the strip of nut meat above the water the growth is vigorous, and if the water surface remains near enough to the strip it is destroyed within eight or ten days. The strip in the water often appeared to be intact when it was not, the mycelium retaining the outline. It was probably destroyed as soon as the strip above.

In hanging drop the conidia begin germinating after three or four hours at room temperature, but many of them require twenty-four hours or more. Seldom more than two cells of a spore germinate, but frequently one cell produces two germ tubes (fig. 46). It often happens that spores are united by a short germ tube. Occa-

sionally four or five conidia are connected in this way (fig. 47), resulting, as is clearly shown by drop cultures, from germination succeeded by anastomosis (fig. 48). In all cultures the dense mycelium collects and retains water enough to germinate the 1- and 2-celled spores, and their germ tubes anastomose readily with the first cells, conidial or hyphal, with which they come in contact. It is often difficult to distinguish between conidiophores bearing conidia and conidia anastomosed to hyphal cells with a short germ tube (fig. 44).

TAXONOMY.—The fungus is a species of *Fusarium* which, according to WOLLENWEBER'S (**34**) scheme of classification, belongs to the section Eupionnotes: chlamydospores present; perithecia unknown; conidia subcylindrical, sickle-shaped; base without pedicel, conical; terminal chlamydospores.

4. ASPERGILLUS DECAY

GENERAL DESCRIPTION.—Brazil nuts attacked by *Aspergillus* may give no external indication of their internal condition except in the most advanced stages of the disease, when the weight of the nut is appreciably lowered. The kernel shrinks, often cracks open, and is always covered with a mass of dark brown spores. The odor of the diseased nut is strongly rancid with a putrid taint; the taste is at first sour, later very bitter. Occasionally nuts that are merely discolored have this same taste. KUHL (**13**) states that Brazil nuts affected with *Aspergillus flavus* Mont. are poisonous, and that the discoloration caused by this fungus is so slight that it does not prevent their being eaten. Both his observations and my own indicate that the disease, although present, may often escape notice, and that it is really far more prevalent than it appears to be under superficial examination. Nuts in advanced stages of the disease, however, occur less frequently than black crust. The mycelium of the fungus penetrates the tissues to the center of the nut, and when there is a central locule, appears as a white mold on the walls of the locule. When the diseased kernels crack open, a mass of spores fills the locular space.

MORPHOLOGY.—The mycelium consists of irregularly branched hyphae which are slightly constricted at the septa (fig. 31). The cells are 20–65 × 3.5–11 μ, with granular contents of a faint greenish

tint. Conidiophores varying from ten to several hundred microns in length arise at irregular intervals from the hyphae. The shortest of these have little or no filamentous part, but consist merely of the head and sterigmata (fig. 28). Sterigmata are also borne singly and in groups of from two to four on the hyphal cells (fig. 27). The heads of the conidiophores measure 10–20 μ in diameter, and the sterigmata, from two to many per head, are 10–12 $\times$ 5–7 μ. The globular, echinulated conidia are of different shades of yellow, and 5–10 μ in diameter, but the predominant size is 7 μ (fig. 29).

CULTURE CHARACTERS.—On cornmeal agar the rate of growth varies from 0.3 to 1.0 mm. daily at room temperature, and after forty-eight hours the central portion of the thallus shows the forming spore clusters in Light-Buff. The spore masses become darker with age until Lemon-Chrome is finally reached. On Brazil nut agar the growth is very similar to that on cornmeal agar, but with a halo 1 to 2 mm. in width, showing the presence of an extracellular, proteolytic enzyme surrounding the thallus. The color of the spore mass at maturity is from Orange-Cinnamon to Mikado-Brown. On nut plugs the growth is rapid, and a gas with the odor of carbon bisulphide is evident. The color of the spore mass is Primrose-Yellow at first, Honey-Yellow to Tawny-Olive at maturity. At the end of two or three months all that remains of the nut plug is a mass of partially disintegrated cell walls in a mass of mycelium.

The growth on autoclaved rice is vigorous, with spore masses forming within forty-eight hours. There is little change in the color of the medium except for the development of a slight greenish-yellow tint below the spore mass. The color of the spore mass changes from Oil-Yellow to Orange-Cetrine. The odor of a 30-day old culture is very like that of cider vinegar. The nut strip above the water is entirely covered with spore masses within three days, but only about one-fourth of it is destroyed before it becomes too dry to support the fungus. A luxuriant growth of mycelium arises from the strip in the water, and usually the strip is destroyed before fifteen days.

TAXONOMY.—A culture of this species was sent to CHARLES THOM, and the following excerpt is taken from his reply dated January 29, 1920:

> The organism belongs to the general group in which we are trying to separate three lines, the *Aspergillus oryzae-flavus* line, the *Aspergillus wentii* section, and the one which has been designated by KITA as *Aspergillus tamari*. This one, from the examination today, would appear to belong to the section containing *A. tamari*. Whether it is safe to identify it under an existing name or not would be doubtful.

5. BACTERIAL DECAY

GENERAL DESCRIPTION AND MORPHOLOGICAL CHARACTERS.—When Brazil nuts are affected by this bacterial decay, the shell is black and greasy, and usually exhales a rancid odor. When the shell is cracked open the remains of the kernel are found as a white mass which ordinarily fills only a small portion of the shell cavity. Microscopic examination of fragments of the refuse shows numerous bacterial spores, but usually no vegetative forms and no fungi. When dilution plates were made from the decayed residue, one spore-bearing organism largely predominated.

The vegetative cells of the organisms in cornmeal broth are rod-shaped, rounded at the ends, vigorously motile, and usually single but often in chains of from two to six individuals. The rods measure 2.5–5.0 $\mu \times 0.8$–1.2 μ. Spores are formed within forty-eight hours in one end of the vegetative cells. When the cells are stained by LOEFFLER'S method, the organism is found to have numerous long, peritrichiate flagella; stained with LOEFFLER'S methylene blue the protoplasm is seen to be granular with from two to four darkened patches which are unevenly distributed, usually giving a banded effect, although often the bands are oblique as well as horizontal (fig. 38). The organism stains readily with methylene blue, Gentian violet, and carbol-fuchsin, but it is Gram negative.

When sterile nut plugs were inoculated with the bacillus from pure culture, they were reduced in about fifteen days to an oily mass which, in all essential characters, was like the remains of the nut kernels in the natural cases of nut decay. Dilution plates made from nut meats that had decayed, following pure culture

inoculation, showed only one type of colony, and this proved to consist of the organism with which the plugs had been inoculated. The organism had no appreciable effect on the nut strip above the water, and the strip in the water was only very slowly decomposed, but strips in cornmeal bouillon were completely destroyed within ten to fifteen days. The organism grows best in the presence of air, as the colonies on all plated media and stab culture show, but deep lenticular colonies (fig. 40), and colonies next to the glass (fig. 39) in agar plates, as well as the faint line of growth along the stab, indicate that it is a facultative anaerobe.

While none of the usual tests for particular enzymes was made, the reactions in different culture media indicate the production of diastase, invertase, rennet, and pepsin. In Brazil nut agar plates there is formed a transparent halo about the colony, and as the opacity of the agar is due to the presence of solid proteid matter (**20**), the halo results from the digesting of these proteids. There is an abundant secretion of the protease which makes the halo, as the diameter of the transparent area is from two to three times that of the colony itself. This enzyme was precipitated as already described, and drops of a water solution of the dried precipitate placed on Brazil nut agar plates. A transparent area as large as the drop of solution was formed in a plate 2 mm. thick in from two to three hours.

The organism seems to be an undescribed one, and a complete description of it will be given in a separate paper.

6. Actinomyces decay

General description and morphology.—Empty shells that are intact and still retain their normal color are occasionally found among Brazil nuts. When these shells are cracked open a characteristic musty odor is evident, and the inner shell wall is seen to be covered with pinkish velvety pustules that are from one to several millimeters in diameter. Water mounts of pieces of a pustule show tenuous, mycelial-like strands, or chains of spores which readily stain with carbol-fuchsin. The filaments are not long but branch, and the mass is so bound together by the branches that it is quite impossible to separate entire filaments from the mass.

The filaments are never entirely straight nor yet very crooked, and chains of spores are usually contained in the free ends (fig. 37). No spirals were found on any of the media. The diameter of the filaments varies from 1.0 to 1.3 μ, and the oblong spores measure 1.6×0.8 μ.

The germination of spores was studied with an oil immersion lens, in a hanging drop prepared as follows. A thin film of synthetic agar was spread on a thin cover-glass, and a loop full of a dilute spore suspension placed on the agar film. This was inverted over a dry Van Tieghem cell. The water soon evaporated, leaving the spores in contact with the agar, where their germination was easily studied and camera lucida drawings made. According to DRECHSLER (9), *Actinomyces* spores produce from one to four germ tubes, "the approximate number being more or less characteristic of the species." This species produces one and two germ tubes which often branch directly on leaving the conidium (fig. 36).

The organism was studied in the manner suggested by CONN (3) and WAKSMAN (33), and the media were made in accordance with directions given by WAKSMAN (33). The following culture characters were noted:

CULTURAL CHARACTERS.—1. Synthetic agar: room temperature, after ten days: growth densely compact but thalli small, at first white, but after ten days Pale Pinkish Buff; aerial mycelium white and dense; soluble pigment none.

2. Calcium malate-glycerin agar: growth spreading and not zonated, bordered by submerged mycelial bands of varying width, pearl white; aerial mycelium short, loose, and pearl white; soluble pigment.

3. Glucose agar: growth luxuriant, color same as in synthetic agar, thallus conspicuously zonated; aerial mycelium white to Pale Pinkish Buff, powdery; soluble pigment none.

4. Glycerin agar: growth densely compact, not zonated, Pale Pinkish Buff; aerial mycelium powdery, white; soluble pigment none.

5. Brazil nut agar: growth rapid, densely compact with wide margin of submerged mycelium, white to Pale Pinkish Buff;

aerial mycelium dense, white; soluble pigment none; enzymatic zone three to four times the diameter of thallus.

6. Cornmeal agar: growth dense but zonated, Pale Pinkish Buff; aerial mycelium powdery; soluble pigment none.

7. Egg albumin agar: growth thin, conspicuously zonated, Pale Pinkish Buff; aerial mycelium powdery, Pale Pinkish Buff; soluble pigment none.

8. Nut plugs: growth vigorous, Pale Pinkish Buff; aerial mycelium powdery, white; medium not completely destroyed, but much shrunken and blackened.

9. Autoclaved rice: growth vigorous, Pale Pinkish Buff; aerial mycelium 2 cm.; almost completely destroyed in sixty days.

10. Potato plugs: growth vigorous, crumpled, Pale Pinkish Buff; aerial mycelium abundant, at first white, later Pale Pinkish Buff; medium with no change in color, much reduced in size in two months.

11. Carrot plugs: growth at first slow, appearing after five days, crumpled and dense, Pale Pinkish Buff; aerial mycelium powdery, at first white, later Pale Pinkish Buff; medium darkened near the growth, no change in color in other regions, much shrunken.

12. Brazil nut bouillon: growth surface pellicle, snow-white; aerial mycelium white, powdery; medium somewhat clarified.

13. Nut strips: growth slight on strip above water, and none on strip in water; no growth on surface of water.

BIOCHEMICAL FEATURES.—The proteolytic enzyme which makes the halo in Brazil nut agar plates was the only one studied, but the growth reactions in different media were taken to indicate the probable production of several other enzymes, diastase and invertase especially. The proteolytic enzyme was isolated by precipitation, as previously described, and its proteolytic power tested by placing drops of a water solution of the dried precipitate on Brazil nut agar plates. Transparent areas the size of the drops developed in from two to three hours, depending upon the thickness of the agar plates.

TAXONOMY.—The organism is an *Actinomyces* which, according to WAKSMAN'S key, belongs in division *B*, "no soluble pigment produced on gelatin or other protein media," and in section *I*,

"species strongly proteolytic; gelatin liquefied rapidly, milk clotted and peptonized rapidly." No species given in this division and section, however, has the characteristics of the one found in Brazil nut shells. It is therefore given the name of *Actinomyces brasiliensis*.

Actinomyces brasiliensis, n. sp.—Straight, branched hyphae 1.0–1.3 μ in diameter; spores borne in chains in free ends of hyphae, oblong, 1.6×0.8 μ; growth Pale Pinkish Buff on all agars except calcium malate-glycerin, on which it is white; zonated on glucose, cornmeal, and egg albumin agars; aerial mycelium on all media, white to Pale Pinkish Buff; no soluble pigment formed.

HABITAT.—Parasitic on kernels of Brazil nuts.

7. PHOMOPSIS DECAY

GENERAL DESCRIPTION.—Only one nut was found affected with *Phomopsis* decay, but because of its striking diagnostic features the fungus was isolated and studied. There was no external indication of the diseased condition, but the kernel of the nut was rich brown, with a few black specks near one end. The odor of the nut was pleasant and the taste agreeable. Stained hand sections showed that the mycelium of the fungus had penetrated into the radicle to considerable depth.

MORPHOLOGY.—The mycelium was tenuous, septate, and at first hyaline, but soon became brown or smoke colored. According to DIEDICKE (**8**), the form of the pycnidia is greatly varied. In the Brazil nut species several of the forms pictured by DIEDICKE were observed, but the one most commonly met with was mammiform, with a wartlike protuberance. The irregular pycnidial cavity so common to the genus was frequently observed, but a regular cavity was the rule. Two forms of spores were present in all pycnidia examined (fig. 50), and as is customary, the *Phoma* type will be designated as *A*, the filamentous as *B* spores. The *B* form did not germinate in hanging drop, a fact supporting the statement made by GROVE (**10**) that these may or may not be spores. When they fail to germinate they are probably what SACCARDO (**25**) took them to be, conidiophores, which according to GROVE "become more curved than when *in situ*." The *A*

spores are oblong-elliptical, hyaline, guttulate, and measure 5–7×1.7–3.5 μ. The *B* spores are filiform, usually hook-shaped, hyaline, continuous, and measure from 17–24.5×2–3.5 μ.

CULTURE CHARACTERS.—The fungus grew well on all media used. On cornmeal agar the thallus was circular and without zonations. A loose aerial mycelium covered the entire thallus, and numerous pycnidia varying in size were scattered over the surface of the plate. The pycnidia appeared simultaneously with the brown color, which was usually noticed after five or six days. On Brazil nut agar the growth was similar to that on cornmeal agar. The clear halo formed in the agar plate barely exceeded the size of the thallus. On autoclaved rice a brown or smoky color, due to the mycelial growth, was noticeable, but no color change occurred in the medium. Nut plugs were soon covered with a brown mycelium which later became almost black. The surface was soon covered with black, wartlike pycnidia, and the entire mass when cut through suggested a dried sponge. The odor was similar to that of very rancid nuts. The fungus made no growth on nut strips above the water, but a dense mass of mycelium, filled with black strands, developed on the strips in the water. These strips retained their form, but the cessation of mycelial growth, which occurred between ten and fifteen days after inoculation, marked the time of nutrient exhaustion.

TAXONOMY.—The fungus is a typical *Phomopsis* which approaches *P. aucubicola* Grove more nearly than any other described species. *A* spores are shorter, and this, coupled with the fact that it occurs on an unrelated host, necessitates describing it as new.

Phomopsis bertholletianum, n. sp.—Pycnidium dark brown, mammiform, with wartlike protuberance, irregular in shape and size, varying from 0.1 to 1.0 μ; conidiophores filiform, hyaline, continuous, 15–20 μ long, often indistinguishable from *B* spores. *A* and *B* spores present, *A* spores oblong-elliptical, hyaline, guttulate, 5–7×1.7–3.5 μ; *B* spores filiform, usually hook-shaped, hyaline, continuous, 17–24.5×2–3.5 μ.

HABITAT.—Parasitic on kernels of Brazil nuts.

8. Bitter rot

Figure 4 shows a part of a Brazil nut affected with bitter rot, and fig. 49 shows spores of the fungus, two of which have conidiophores attached. Neither the spores nor the mycelium was viable, and time prevented more than a superficial examination being made. The fungus is apparently a *Myxosporium*.

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EXPLANATION OF PLATES VIII–XII

All drawings were made with camera lucida.

PLATE VIII

FIG. 4.—Part of Brazil nut kernel affected by bitter rot; ×1.

FIG. 5.—Thalli of *Actinomyces brasiliensis* n. sp. on cornmeal agar; ×¾.

FIG. 6.—Thallus of *Actinomyces brasiliensis* n. sp. on cornmeal agar; ×2½.

FIG. 7.—Colony of *Bacillus* from Brazil nut on cornmeal agar; ×2.

FIG. 8.—*Pellioniella macrospora* n. sp. in autoclaved rice; tube on right 20 days old; tube on left 10 days old; ×1.

FIG. 9.—Brazil nut 30 days after inoculation with *Pellioniella macrospora* n. sp.; nut plug at top marks place of inoculation, and along two edges inner seed coat removed to expose blackened endosperm; ×1.

FIG. 10.—Thallus of *Actinomyces brasiliensis* n. sp. on Brazil nut agar, surrounded by transparent area in which proteids have been digested owing to secretion of proteolytic enzyme; ×1½.

FIG. 11.—Thallus of *Pellioniella macrospora* n. sp. on cornmeal agar: three zones: (1) scarcely visible outer zone of white; (2) zone of nearly same width that is green in growing thallus; (3) inner black circle; ×¾.

FIG. 12.—*Actinomyces brasiliensis* n. sp. on potato plug after 10 days; ×1.

PLATE IX

FIG. 13.—Paraphyses, immature conidia, and conidiophores of *Pellioniella macrospora* n. sp.; ×500.

FIG. 14.—Conidia-like cells of hyphae of *P. macrospora* n. sp. from diseased tissue of Brazil nut; ×500.

FIG. 15.—Mature, immature, and transitional stages in development of conidia of *P. macrospora* n. sp.; ×500.

FIG. 16.—Germination of immature conidia of *P. macrospora* n. sp., planted in same hanging drop with mature conidia shown in figure 18; ×500.

FIG. 17.—Germination of mature conidia of *P. macrospora* n. sp.; germ tubes from one to two hours longer in emerging than those of immature conidia shown in figure 17; ×500.

FIG. 18.—Hypha of *P. macrospora* n. sp., showing most common type of cell; ×500.

FIG. 19.—Hypha of *P. macrospora* n. sp. from near pycnidium; ×500.

FIG. 20.—Conidia-like cells of hyphae of *P. macrospora* n. sp., taken from culture in autoclaved rice; ×500.

FIG. 21.—Section of pycnidium of *P. macrospora* n. sp., enlarged about 450 diameters.

FIG. 22.—Hyphae of *P. macrospora* n. sp., showing two types of cells; ×500.

PLATE X

FIG. 23.—Conidia of *Cephalosporium bertholletianum* n. sp.; ×500.

FIG. 24.—Hyphae, conidiophores, and spore masses surrounded by water drops, *C. bertholletianum* n. sp.; ×500.

FIG. 25.—Germinating conidia of *C. bertholletianum* n. sp., two hours after planting; ×500.

FIG. 26.—Germinating conidia of *C. bertholletianum* n. sp., twenty hours after planting; ×500.

FIG. 27.—Hyphae of Brazil nut *Aspergillus* bearing sterigmata; ×500.

FIG. 28.—Hypha of Brazil nut *Aspergillus* bearing short stalked conidiophores; ×500.

FIG. 29.—Conidia of Brazil nut *Aspergillus;* ×500.

FIG. 30.—Mature conidiophores of Brazil nut *Aspergillus;* ×500.

FIG. 31.—Hyphae showing branching habit and anastomosis, Brazil nut *Aspergillus;* ×500.

FIG. 32.—Early stages of conidiophores of Brazil nut *Aspergillus;* ×500.

PLATE XI

FIG. 33.—Microtome section of normal nut kernel showing tissues named in order, beginning at top: endosperm, epidermis, cortex, procambium, and medulla; ×500.

FIG. 34.—Microtome section of Brazil nut affected by *Pellioniella macrospora* n. sp., showing dense mycelial growth in endosperm region; ×1000.

FIG. 35.—Microtome section of Brazil nut affected by *P. macrospora* n. sp., showing relation of fungus to host tissues; ×500.

FIG. 36.—Germinating conidia of *Actinomyces brasiliensis* n. sp.; ×1000.

FIG. 37.—Hyphae and conidia, *Actinomyces brasiliensis* n. sp.; ×1000.

FIG. 38.—Vegetative cells of Brazil nut bacillus; ×1000.

FIG. 39.—Colony of Brazil nut bacillus growing near glass in cornmeal agar plate; ×50.

FIG. 40.—Deep colony of Brazil nut bacillus n. sp.; ×50.

FIG. 41.—Surface colony of Brazil nut bacillus n. sp.; ×50.

PLATE XII

FIGS. 42–49, and 51 are of a *Fusarium* which causes dry rot of Brazil nuts.

FIG. 42.—Conidial variation and anastomosis; ×500.

FIG. 43.—Typical hyphae showing difference in size and branching habit; ×500.

FIG. 44.—Hyphae bearing single conidiophores and conidia anastomosed to hyphal cells by germ tube; ×500.

FIG. 45.—Terminal chlamydospores; ×500.

FIG. 46.—Germinating conidia in hanging drop culture; ×500.

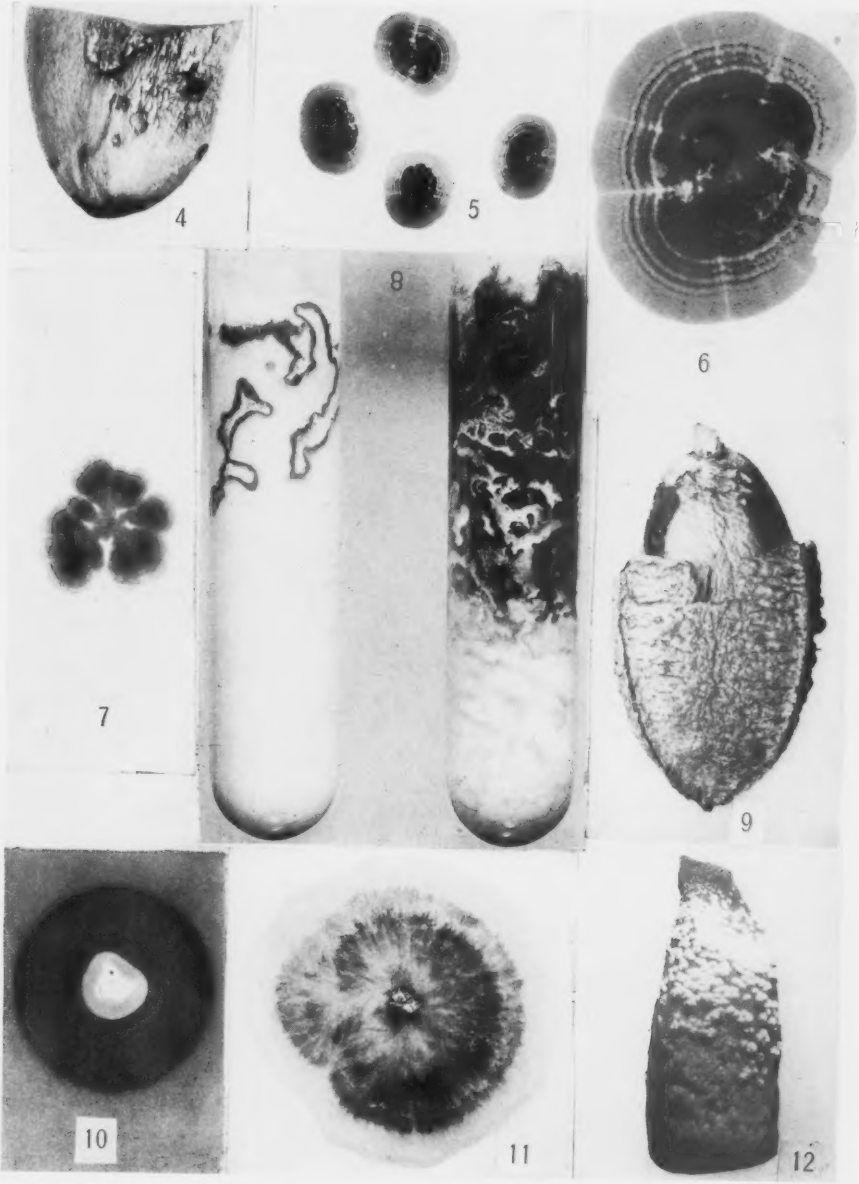
FIG. 47.—Anastomosing conidia and hyphae from culture plates; ×500.

FIG. 48.—Anastomosis of germinating conidia in hanging drop; ×500.

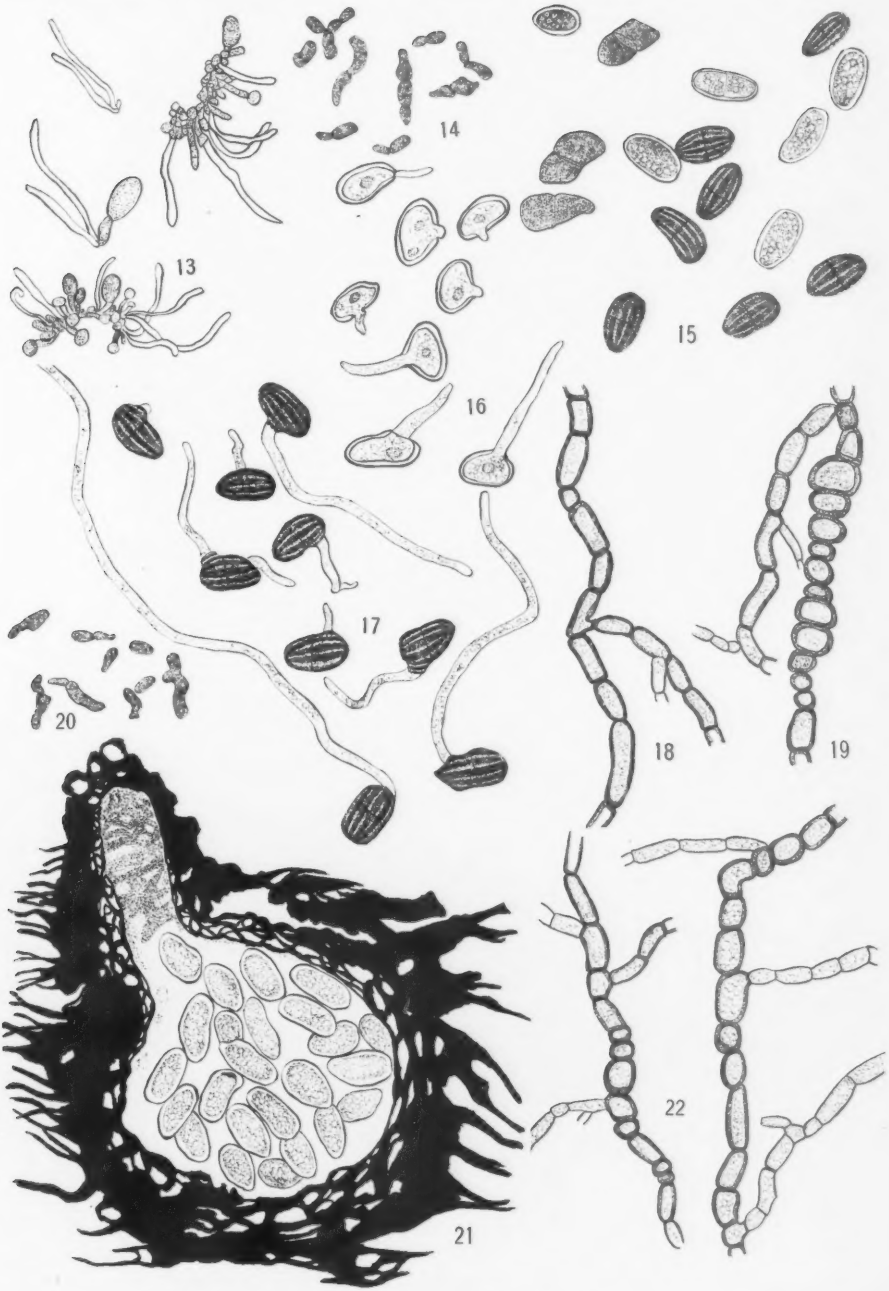
FIG. 49.—Conidia and conidiophores of bitter rot fungus, taken from pustules on diseased kernel; ×500.

FIG. 50.—*Phomopsis* conidia, two having germ tubes attached; ×500.

FIG. 51.—Hyphae of *Fusarium* from washed agar plates; ×500.



SPENCER on BRAZIL NUTS



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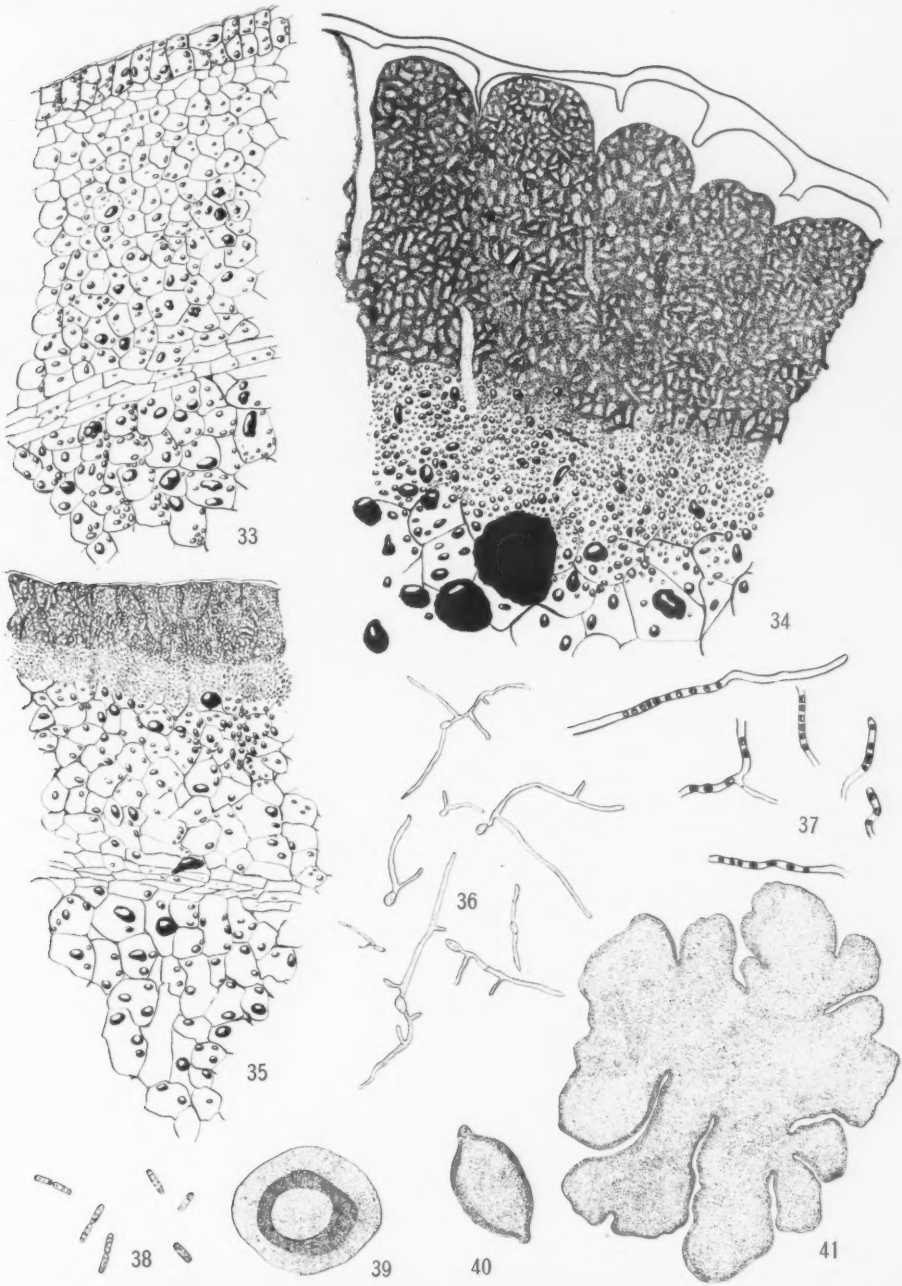

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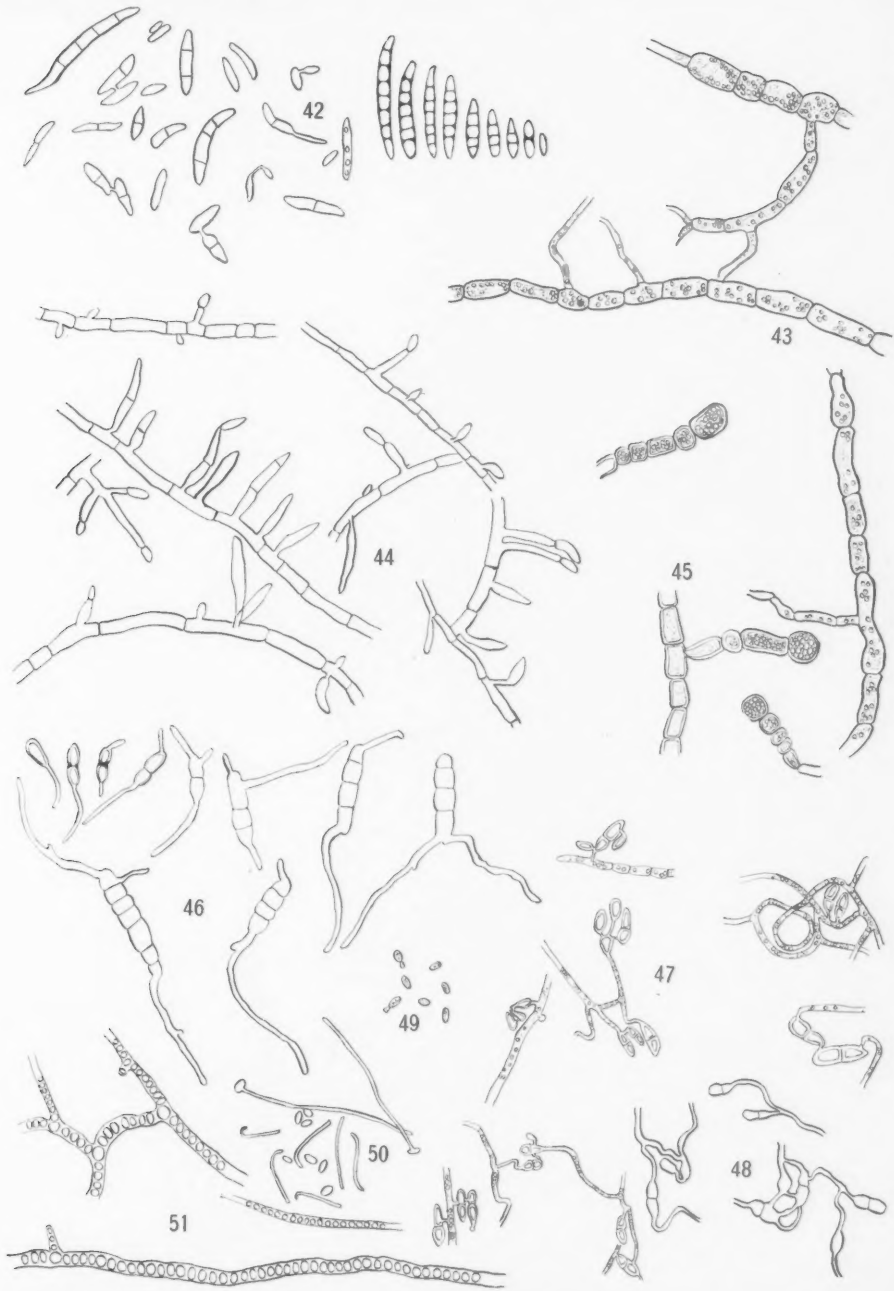


SPENCER on BRAZIL NUTS

GROWTH RINGS IN A MONOCOTYL

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 285

CHARLES J. CHAMBERLAIN

(WITH SIXTEEN FIGURES)

The principal object of this paper is to announce the discovery of growth rings in a monocotyl, but some observations upon growth rings in other plants may not be out of place. The most familiar example of periodic growth is seen in the annual rings of Gymnosperms and dicotyls; but even when there is a strong tendency to form only one ring every year, there are numerous variations, especially when the rings are very wide.

In *Melia azedarach* the annual rings are often more than a centimeter in width, but it is common to find in each season's growth a dozen or more secondary rings which are easily seen with the naked eye. The part of the ring formed in the spring and summer is quite sharply differentiated from that formed in the autumn, and it is in this autumn wood that the secondary rings are most conspicuous. In the spring and summer wood the few rather indefinite secondary rings are due to varying proportions of tracheae and tracheids. The tracheae of summer wood are not very different from those of early spring; while in the autumn wood the larger cells merely start to develop into tracheae. They have transverse walls, which in some cases begin to break down, but here the development ends. The tracheids of the autumn wood are numerous and very thick-walled, so that this part of the ring is extremely hard. It is evident that the secondary rings are due to periodic acceleration and retardation of growth, which causes them to show some of the features characteristic of ordinary annual rings.

Casuarina tenuissima affords another instance of numerous rings. A shoot about 3 mm. in diameter and only a few weeks old showed five or six well marked rings, due to an alternation of tracheae and tracheids. The plant from which the shoot was taken was

growing in the greenhouse, and the number of rings corresponds, roughly, to the number of times the plant was watered thoroughly. Several years ago, in the neighborhood of Jalapa, Mexico, where it is rather rainy throughout the year, a species of *Piper* was noticed which showed no growth rings; while the same species, a few miles farther east, where there is a sharp alternation of wet and dry seasons, showed the anticipated rings. These are examples of immediate response to rather slight changes in conditions. At the other extreme are plants which show no response to seasonal conditions, but nevertheless are susceptible to stronger stimuli.

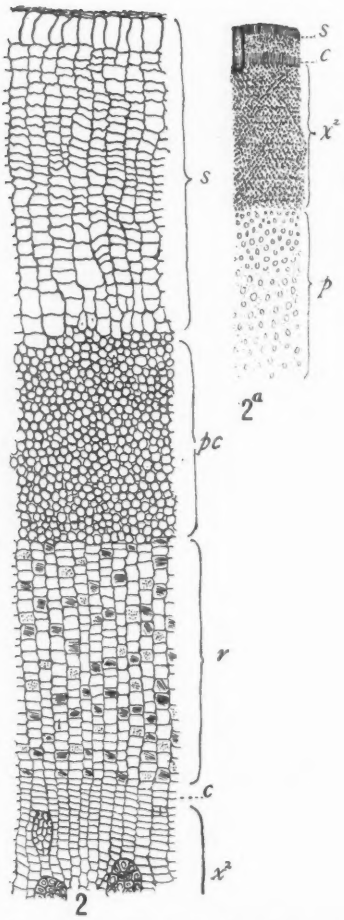
Interesting growth rings which do not mark the number of years, but correspond to longer intervals, are found in the cycads. *Dioon edule*, after a period of coning or after damage by fire, loses all its leaves and goes into a prolonged resting stage which may last for several years. When it resumes activity and produces a new crown, a vigorous growth of wood takes place, with the formation of large tracheids, which, following the small tracheids of the nearly exhausted condition, produce a ring having the characters of an ordinary annual ring in Gymnosperms. These prolonged resting periods occur at long intervals, so that the number of rings would be of slight value in estimating the age of a plant. A section of the trunk of a specimen of *D. edule* 1.5 to 2 m. in height would enable one to estimate the interval between successive growth rings, since the approximate age of the plant could be determined; but at present it is not easy to secure such a section. In *D. spinulosum* the rings look like those of *D. edule*, but a ring is produced with the formation of every crown of leaves. Since crowns in this species are usually formed every other year, the number of rings indicates about half the age of the plant. In these cases the ring is a response to a change in conditions, but a very decided change is necessary to produce the result.

It has long been known that some arborescent monocotyls, like *Dracaena*, *Yucca*, and *Aloe*, produce a distinct zone of secondary tissue surrounding the primary and derived from a meristematic region showing the characters of cambium. In 1912, while studying cycads in South Africa, I cut into a large plant of *Aloe ferox*

to get material for demonstration purposes, and it was surprising to find growth rings so conspicuous that they could be seen where the stem was cut with an ax. Pieces to show both primary and secondary structures were preserved in formalin, and later *Aloe pleuridens*, *A. ciliaris*, and *Dracaena Hookeriana* were collected for comparison.

FIG. 1.—*Aloe ferox* at Cathcart, South Africa, January 1912; about 3 m. in height.

Aloe ferox in the field presents a picturesque appearance, looking as if an *Agave* had developed a tall trunk (fig. 1). It is associated with other xerophytic plants as bizarre as itself, among them tree forms of *Euphorbia* more than a dozen meters in height, species of *Encephalartos*, and others not so large but equally peculiar. Most of the material was collected near Grahamstown, South Africa, in January 1912, from a stem 15 cm. in diameter and about 3 m. high. In transverse section the zone of secondary xylem



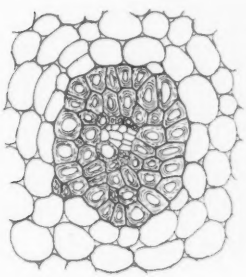
FIGS. 2, 2a.—Fig. 2, *Aloe ferox*, transverse section of part of stem: *s*, secondary cortex; *pc*, primary cortex; *r*, outer region of cells cut off by cambium and containing numerous raphides; *c*, cambium; x^2, region of secondary bundles from inner region of cells cut off by cambium; ×40; fig. 2a, *Aloe ferox:* part of transverse section of stem, natural size; fig. 2 shows part included in rectangle at upper left corner: *s*, secondary cortex; *c*, cambium; x^2, region of secondary bundles; *p*, primary polystelic region.

was 2 cm. and the cortex 4 mm. in width; so that the central region, nearly 10 cm. in diameter and consisting of primary structures, gave the whole section somewhat the appearance of a large pith surrounded by a narrow zone of wood and a scanty cortex.

The general topography of a small portion of a transverse section, natural size, is shown in fig. 2a. In the primary region (*p*) the bundles are large and scattered, as in a cornstalk; while in the zone of secondary growth (x^2) the vascular bundles are so regularly arranged, that to the naked eye they form a pattern like the chasing on a watch. The cambium (*c*), which is giving rise to secondary bundles, the secondary cortex (*s*), and some of the primary cortex between these two zones of secondary growth, are also visible to the naked eye.

The phellogen, with the secondary cortex produced by it, the inner cambium with its derivatives, and also the primary cortex (*pc*) between the two secondary products, are shown in fig. 2. The walls of the secondary cortex are

slightly thickened, but thoroughly suberized. Beneath the secondary cortex is the primary, consisting of loose rounded cells with



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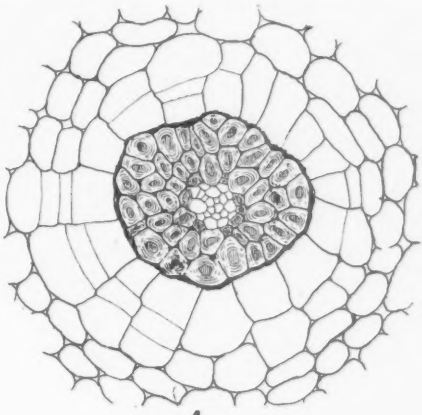

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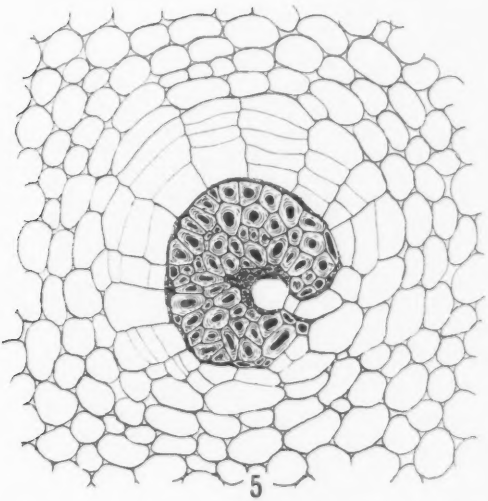

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FIGS. 3–5.—FIG. 3. *Aloe ferox:* normal bundle of primary polystelic region; ×100; fig. 4. *Aloe ferox:* bundle of primary polystelic region, showing clogged lumen of tracheids; some of cells immediately surrounding bundle becoming meristematic; ×100; fig. 5, *Aloe ferox:* more advanced condition than in fig. 4; ×100.

cellulose walls, and between the primary cortex and the primary polystelic region is the zone which contains the secondary vascular bundles and shows the growth rings.

The primary polystelic region, in transverse section, looks somewhat like an immense cornstalk, with large bundles toward the center and smaller ones at the outside; but the structure of the individual bundles is very different from that in corn, for the bundles in *Aloe* have no sheath and most of them are completely amphivasal. There seem to be two types of vascular bundles in

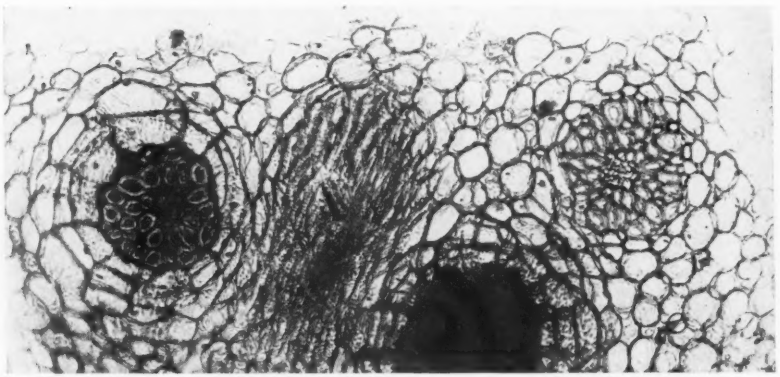
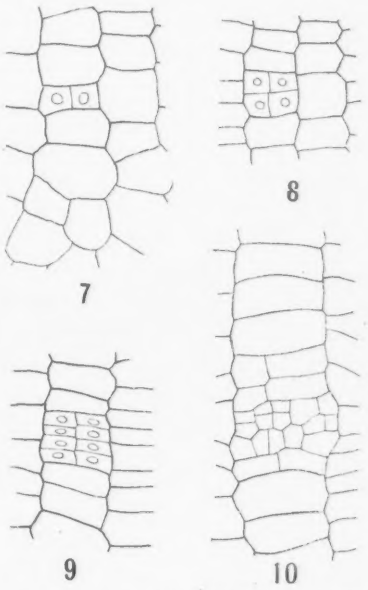


FIG. 6.—*Aloe ferox:* showing both types of primary bundles; ×100

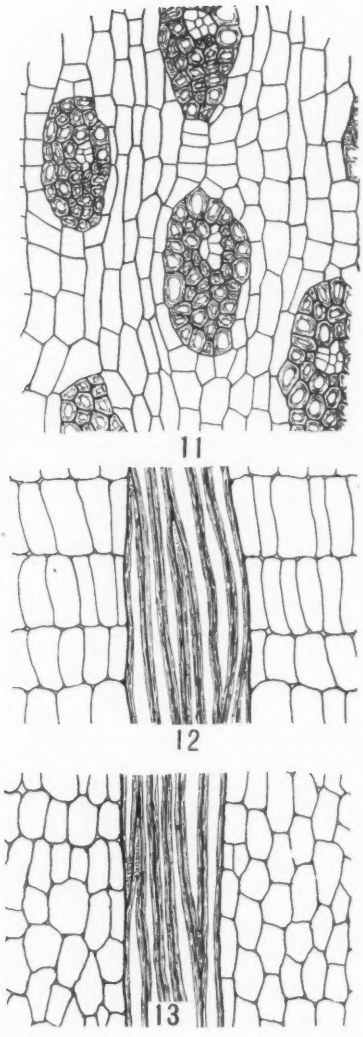
this primary region. In one type the bundles have normal xylem and phloem, except that the phloem has very few companion cells (fig. 3). The other type is peculiar. The phloem begins to disorganize and finally disappears, while the lumen in both tracheids and vessels becomes clogged with the same material found in the disorganizing phloem. Some of this material can also be seen surrounding the bundle itself. An early stage, shown in fig. 4, and a later stage, shown in fig. 5, are characteristic. In the latter figure the contents of the xylem cells are much denser and the phloem cells have become almost indistinguishable, while the adjacent thin-walled parenchyma is crowding into the space left vacant by the disorganizing phloem.

Another peculiar feature of the bundle with disorganizing phloem is the appearance of vigorous meristematic activity in the cells surrounding the xylem. These cells behave like a cambium, so that rows consisting of as many as eight cells may be formed (figs. 4, 5). If differentiation should take place, we should expect to find a xylem zone and, perhaps, phloem surrounding the primary bundle; but development stops soon after the stage shown in fig. 5, before any lignification can be detected. The distribution and general appearance of the primary bundles are shown in fig. 6.



FIGS. 7–10.—*Aloe pleuridens:* four early stages in development of secondary bundle; ×100.

That the secondary growth in some monocotyls, like *Yucca*, *Dracaena*, and *Aloe*, results from meristematic activity is well known. The piece of a transverse section of *Aloe ferox*, natural size (fig. 2*a*), already referred to, and a somewhat magnified view of the origin of secondary structures (fig. 2), show the position of the structures to be described. The phellogen is evidently hypodermal in origin, and it builds up a limited amount of secondary cortex, with rectangular cells in regular rows abutting upon the smaller spherical cells of the primary cortex. The cambium which gives rise to the vascular structures is pericyclic in origin, and, as seen in transverse section, gives rise to long rows of cells. The cells on the outer side of the cambium undergo comparatively little differentiation; they enlarge to about twice the size of the cambium cells, and many of them become almost entirely filled with needle-shaped crystals of calcium



FIGS. 11–13.—*Aloe ferox:* fig. 11, transverse section of secondary bundle; fig. 12, longitudinal radial section; fig. 13, longitudinal tangential section; ×100.

oxalate; but the cell walls thicken very little and retain the cellulose reaction (fig. 2 *r*.)

The cells formed centripetally from the cambium give rise to the secondary woody structures which show the growth rings. The development of the bundle was not studied very thoroughly in *Aloe ferox*, but the early stages are about the same as in *A. pleuridens*. As seen in transverse section, a cell of the row produced by the cambium divides, the two resulting cells divide, and the process continues until forty or fifty cells are formed (figs. 7–10). Differentiation of the young cells of the vascular strand begins to take place before the full number of cells has been reached. These bundles are completely amphivasal and there is no sheath of thick-walled cells. The phloem is scanty and companion cells are rare. There is no degeneration or clogging of the lumen in the secondary bundles, and there is no meristematic activity in any of the surrounding cells, like that which characterizes many of the bundles of the primary cylinder. Since the

bundle is completely amphivasal, there is no cambium between the xylem and phloem, like that found in the primary bundles of many monocotyls (fig. 11).

The xylem consists almost entirely of tracheids with bordered pits and with walls so thick and hard that sectioning is difficult. The cells cut off from the inner side of the cambium and not taking part in the formation of the bundles keep, more or less perfectly, their linear arrangement. They are short, somewhat rectangular in radial view, and are arranged in very definite rows (fig. 12). The tangential arrangement is not so regular (fig. 13). While they thicken only a little, they become thoroughly lignified and extremely hard, so that they add to the difficulty of cutting sections. They are marked by numerous small simple pits.

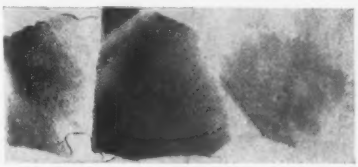


FIG. 14.—*Aloe ferox:* three thick transverse sections of stem; natural size.

The growth rings constitute the most striking feature of the stem. DE BARY, in his *Comparative anatomy of vegetative organs of the phanerogams and ferns*, remarked that, while there seemed to be no reason why growth rings should not be formed in woody monocotyls, none had ever been observed. An examination of the literature of vascular anatomy failed to yield any account of such rings; but, to make certain that nothing had been overlooked, I wrote to Professor JEFFREY, and he not only informed me that such rings had never been reported, but also gave some suggestions which greatly facilitated the investigation.

To the naked eye the growth rings are obvious, but under a 16 mm. objective no one would suspect their presence. In *Dioon*, where growth rings are obvious to the naked eye but not so conspicuous under the microscope, the rings are due to the fact that cells formed at the close of a growth period are somewhat smaller and have thicker walls than those formed when growth is resumed. In *Aloe ferox* the explanation is not so evident. An examination of fig. 14, showing three thick transverse sections, indicates that the rings can be seen, even in a half-tone reproduction.

The rings can be seen more clearly by looking across the figure from nearly the level of the paper. The negative was made twice the size of the section, and the illustration reduced to natural size. The same sections without reduction are shown in fig. 15. The appearance, under a low magnification, is shown in fig. 16, which includes six of the growth rings. The rings are not at all conspicuous, and the number of rings probably could not be counted in the illustration. Even with the position on the rings marked with the numerals 1–6, they are not easily identified. Two structural features cause a ring. At the close of the growing period a few

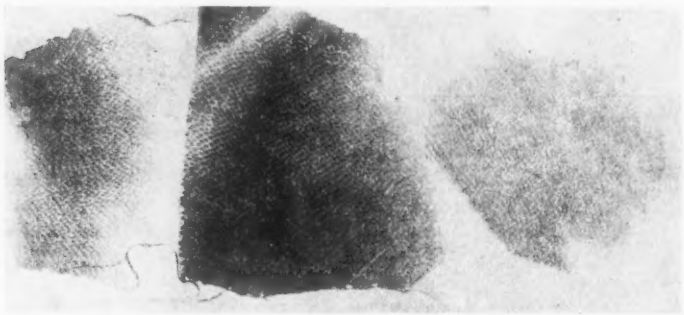


FIG. 15.—*Aloe ferox:* from same negative as fig. 14; ×2

smaller bundles at irregular intervals are probably responsible, but the principal cause is that the parenchyma cells formed at the close of a growing period are slightly smaller and have slightly thicker walls.

I wrote to Professor SCHÖNLAND, Director of the Albany Museum at Grahamstown, South Africa, and to Mr. E. E. GALPIN, formerly of Queenstown but now of Naboomspruit, Transvaal, South Africa, inquiring about climatic conditions in the localities from which the material was secured. Professor SCHÖNLAND, to whom I am also indebted for material of *Aloe ferox*, wrote as follows:

> There are two maxima of rainfall, in October and November, and in March and April; but this comes out only when the averages of a number of years are worked out and give a wrong picture of the relation of the flora to our rainfall. It is true that the winter, from the middle of June to the middle of September, is generally dry; but I have known good rains in these three months. Last year (1920) the October-November rains failed us; we had

good rains at Christmas, then drought to the middle of March, and then good soaking rains. The yearly amount of rainfall is often very interesting. In 1899 we had a serious drought up to Christmas, and then 12 inches in 24 hours. The long and short of it is that we live in what might be called the fag end of the summer rain area; but it can best be described as an area of uncertain rains.

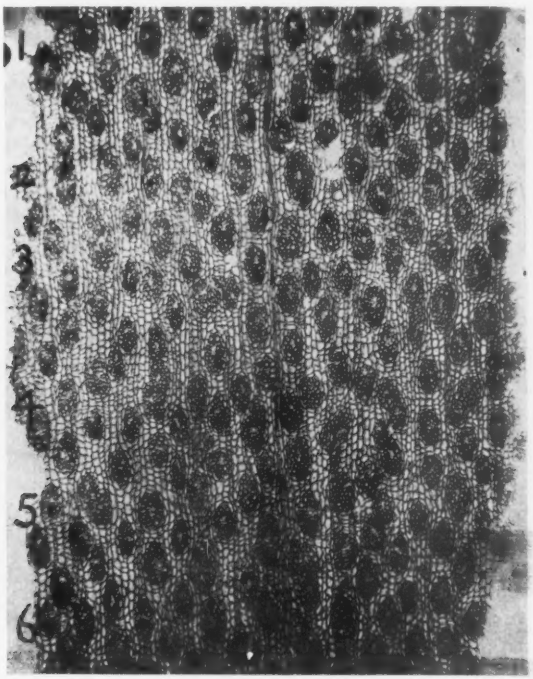


FIG. 16.—Small portion of region of secondary bundles, including six growth rings, numbered 1–6; ×10.

Mr. GALPIN replied as follows:

Around Grahamstown and the coastal districts generally, the seasons are very equitable, with no winter frosts and the rainfall fairly equally distributed throughout the year. Like the whole of South Africa, they have their lean years and their fat years, with deficient or generous rainfall, as the case may be. Added to this, at long but irregular intervals, perhaps a quarter of a century, more or less, for reasons as yet quite unexplained, there may come a period of prolonged drought or of exceptional rains. These remarks apply to Cathcart as well, but in that district, with an altitude of about 4000 ft., the seasonal changes are greater, with hot summers and frosty nights in winter.

The rainfall is also very much greater in summer than in winter, although the difference between dry and wet seasons is not so marked up here, nearer the tropics. They get a certain amount of rain during the winter months, while at Naboomspruit, with an annual rainfall of 25 inches, usually not a drop falls from the end of April to the beginning or even the end of October.

These two accounts, written by botanists who have made a prolonged study of the South African flora, show that the climatic conditions in the region where *Aloe ferox* grows are somewhat erratic. Large specimens were seen at Junction Farm in the Transkei, near Cathcart, and the negative from which fig. 1 was made was taken on the Windvogelberg, overlooking the town of Cathcart; but no material was collected. Judging from Mr. GALPIN'S account, specimens from the Windvogelberg would show more sharply marked rings than one would be likely to find in plants from the Grahamstown region where this material was collected. Both accounts, however, would lead one to expect the irregularities which appear in the rings of material collected near Grahamstown. Irregularities may be seen in figs. 14 and 15, and in fig. 16, where the position of the six rings is marked by the numerals 1–6.

Whether other species of *Aloe* would show rings or not could be determined very easily by one who is within reach of material. A few slides of *A. pleuridens* show a couple of faint rings. *Dracaena Hookeriana*, collected at East London, less than 100 miles south of Cathcart, shows secondary wood but no growth rings. It would be interesting to see the condition in *Aloe Bainesii*, the trunk of which may reach a diameter of a meter in less than thirty years. A specimen of *Yucca* with a zone of wood a centimeter in diameter, growing in the greenhouse, showed no growth rings; but such rings could hardly be expected in a greenhouse, where conditions are so uniform. One wonders whether the failure to find growth rings in woody monocotyls may not be due to the fact that they are mostly tropical and subtropical, out of the University zone, so that observations are likely to have been made upon greenhouse material. That there are growth rings in *Aloe ferox* is beyond question, and this is believed to be the first account of such rings in any monocotyl.

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INVASION OF VIRGIN SOIL IN THE TROPICS[1]

DUNCAN S. JOHNSON

(WITH TWO FIGURES)

This note is concerned with the revegetation of a tropical valley which was denuded of plants by a flood and later filled with detritus from a landslide. Acknowledgments are due to Messrs. H. A. GLEASON, WILLIAM HARRIS, M. A. HOWE, E. P. KILLIP, W. R. MAXON, and PERCY WILSON for the identification of plants collected in the Cascade Valley; to E. P. KILLIP and WILLIAM SEIFRIZ for taking photographs of the valley; and to JONAS WALKER, a Jamaican collector, for gathering plants growing in the valley in December.

The Blue Mountain region of Jamaica was subjected, in November 1909, to several days of nearly continuous torrential rains, such as apparently occur there only once or twice in a century. On November 8, 1909, there was a rainfall of 18.3 inches in 24 hours at the Cinchona Station, and this downpour continued into the next day, until 27 inches had fallen. The rainfall was undoubtedly heavier still on the higher peaks of the Blue Mountains which drain into the valley under discussion.

The floods arising from these tremendous rains caused striking changes in the topography, and in the plant covering of many considerable areas on both the north and the south sides of the Blue Mountains. In the first place, many small streams rose two or three meters above the normal level, and scoured their rocky banks clean of vegetation, aside from larger trees, for many meters on either side. In the second place, there were landslides from the wooded mountain sides, and especially from the cultivated coffee fields, which completely carried away soil and vegetation from scores of acres on the south side of the mountains. These landslides not only left great scars, showing the bare rock on the formerly tree-covered mountain sides and in the coffee fields

[1] Botanical contribution from the Johns Hopkins University, no. 70.

lower down, but they also filled in whole valley bottoms with the rock and gravel washed down from above. The amount of water and of débris carried with it was sufficient to wash away or bury out of sight most of a large and substantially constructed stone and concrete "coffee works" near the Cascade River.

The effect of the flood and landslides on the topography and vegetation of the valley of the Cascade River, a normally small mountain stream, located about three miles east of the Cinchona Botanical Station, was briefly described in a note published in 1910.[2] At that time, which was but six months after the flood, the floor of this valley was still a barren waste, covered with pebbles and broken rock fragments of all sizes, ranging from that of a pea up to bowlders a meter in diameter. The only plants evident at this time were a few widely scattered seedlings of *Bocconia frutescens* and still fewer seedlings of half a dozen other dicotyledons, such as grow on the hills beside the valley. The largest of these plants were only 2 or 3 dm. high. In other words, the valley bottom, which in 1903 and 1906 I had seen covered with a forest consisting of large trees together with dozens of types of shrubs and herbs, was in 1910 an all but absolute desert. The forest had been completely washed away or buried, and there was left a truly virgin soil, with no trace of humus, which bore but the barest sprinkling of young seedlings.

After studying the conditions in this and other valleys in 1910, and taking into account the abundant rainfall and frostless climate of the region, it was concluded that the floor of the Cascade Valley would probably be recovered with a dense vegetation, although perhaps not with a fully developed forest, in a score or two of years. It was realized, of course, that many of the forest plants, being dependent on an abundant humus, would not find satisfactory conditions there for many years, because of the slowness with which this type of soil is developed.

On a trip to Jamaica, in July 1919, I again visited the Cascade Valley, and expected to find that, during the nine years that had elapsed, the few plants that were starting on the newly deposited gravel in 1910 had multiplied greatly, and that many new species

[2] Jour. New York Bot. Gard. 11:273. 1910.

would be establishing themselves among those first invaders. Many of the possible invaders of the valley, found on the neighboring hills, have a long growing season. There are some species that grow actively from February to September, while still others grow practically throughout the whole year.[3] Because of this long growing season and the possibility of some humus washing down from the surrounding hills, it was assumed that by 1919 the soil of the valley floor would be well hidden by a plant covering.

FIG. 1.—Looking east across Cascade Valley, showing sparse vegetation, which sparseness would be still more evident if viewed from above.

My surprise was great, therefore, when I found hardly more than a tenth of the gravelly bottom of the Cascade Valley hidden by plant foliage. The soil between these leafy plants, it is true, was not absolutely bare. There were a few very small patches of lichens and mosses. There was also a chroococcaceous alga, *Gloeocapsa magma*, which formed smooth encrustations often several square decimeters in extent on the pebbles and bowlders. This alga is present not only near the streams but across the whole floor of the valley. When dry *Gloeocapsa* has a rather dirty or chocolate brown color, but when wet it becomes a glistening velvety layer of a dark maroon color. It evidently thrives on these bare

[3] SHREVE, F., Publication no. 199, Carnegie Inst. Wash. pp. 51–52. 1914.

rocks, although they may often be exposed to a scorching sun for many hours daily and be without rain for days or even several weeks together. There is a copious dew in the valley each clear night, however, while on cloudy nights the fog probably condenses on the rocks and plants of its floor. It is likely that *Gloeocapsa* may thus be able to carry on photosynthesis and growth for some hours each day, even without rain.

The bareness of the valley bottom recalled that of the more barren of the stony deserts of Arizona as they appear in early summer. The general aspect of this valley differed from that of these deserts in the absence of cacti and of all larger woody plants. No plants of this valley exceeded a meter or two in height except where, at the very edges of the valley, considerable top soil, that had washed down from the hillsides, afforded better conditions for plant growth. Here several species of shrubs grew to two or three meters in height, and in wetter soil considerable stands of *Arundo* sp. had established themselves (fig. 1, *x*). The shrubs and cane together made a conspicuous verdant border to the generally desert-like valley floor.

When the floor of the valley, especially the portion along the trail from the junction of the Cascade and Green rivers to Farm Hill Coffee Works, was more carefully examined, the scattered vegetation was found to include the following plants:

ALGAE

Gloeocapsa magma (Breb.) Kütz.

PTERIDOPHYTA

Dryopteris oligophylla Maxon
Blechnum occidentale L.
Gymnogramme tartarea (Sw.) Desv.
Trismeria trifoliata (L.) Diels
Pityrogramma calomelaeana (L.) Link
Pteris longifolia L.
Aneimia adiantifolia (L.) Sw.

DICOTYLEDONEAE

Piper sp.? (shrub or tree)
Pilea microphylla L. (Liebm.) (annual to perennial)
Iresine celosioides L. (half shrubby)
Begonia acuminata Dryand. (half shrubby)
Asclepias curassavica L. (perennial herb)
Asclepias nivea L. (perennial herb)
Philibertella clausa (Jacq.) Vail (shrubby vine)
Duranta plumieri Jacq. (shrub 2-3 meters)
Verbena bonariensis L. (perennial herb)
Solanum torvum Sw. (half shrubby)
Maurandia scandens A. Gr. (shrubby vine)

	Ageratum conoyzoides L. (annual)
	Ageratum houstonianum Mill. (annual)
MONOCOTYLEDONEAE	Vernonia acuminata Less. (half shrubby)
Arundo (saccharoides Gr. ?)	Vernonia permollis Gleason (half shrubby)
	Mikania scandens L. (Wild.) (shrubby vine)
	Eupatorium triste DC. (half shrubby)
	Baccharis scoparia Sw. (shrubby)
	Pluchea odorata L. (Cass.) (half shrubby)
	Bidens incisa Ker. (annual)
	Senecio discolor (Sw.) DC. (shrubby)

There were thus seven species of ferns, of which *Dryopteris oligophylla*, *Blechnum occidentale*, and *Aneimia adiantifolia* were rare, less than a score of each being seen where we crossed the valley. *Pityrogramma calomelaena* and *Gymnogramme tartarica* were more frequent; while *Trismeria trifoliata* was represented by dozens of specimens in the moister soil, and of *Pteris longifolia* there were still more numerous clumps in the drier spots along the trail across the valley. From the size of many of the fern plants seen it seems clear that they have been established for some time. In the cases of *Gymnogramme* and *Trismeria*, where fronds a meter high were seen, it was hard to believe that such plants could have arisen from a prothallus in nine years. Yet they must have done so unless it is assumed that old rhizomes have persisted in the soil to push up through the gravel, or that pieces of rhizomes have been washed down by the flood of 1909 or subsequent lesser ones. The first supposition seems negatived by the fact that no ferns were seen in 1910, six months after the flood, and also by the fact that each clump of a fern consists of but one or a few branches and leaf clusters. This latter feature tends to confirm the impression gained from the character of the soil, namely, that these ferns have started *in situ* from prothallia.

All the seed plants found in the valley, except *Arundo* along the stream at the foot of the cliff, were dicotyledons. By far the most important of these was the composite *Vernonia permollis*. Scores of clumps of this, from quite young plants up to those 2 m. high, were found scattered across the valley. They grew beside the larger rocks and often also formed rather definite rows along

the small dry gullies, which during the rainy season drain the raised middle of the valley floor that lies between the main stream on the west and the branch stream that comes in from the east. This ironweed is the most prominent plant of the valley, not only because of its abundance but also from its size. It is this plant, for example, that forms the major component of the clumps shown in fig. 1. The three more prominent plants after *Vernonia permollis* are *Bocconia frutescens* (already grown to 2 or 3 m. in height), *Solanum torvum Sw.* (often 2 m. high), and *Vernonia acuminata*

FIG. 2.—Looking north over upper Cascade Valley, showing scars left on south side of Blue Mountains by landslides.

(about 2 m). These larger plants are sometimes mingled with the *Vernonia permollis*, although much fewer than the latter, but may also be scattered sparingly by themselves over the valley floor.

Of the less prominent seed plants of the valley, some fifteen species were found. These, with their relative abundance, are: *Piper* sp.? (two or three young plants), *Pilea microphylla* L. (Liebm.) (rather frequent), *Iresine celosioides* L. (sparse), *Begonia acuminata* (very few), *Asclepias curassavica* L. and *A. nivea* L. (both infrequent), *Philibertella clausa* (Jacq.) Vail. (a dozen plants seen), *Duranta plumieri* Jacq. (half a dozen plants), *Verbena bonariensis* L. (few), *Solanum torvum* Sw., *Maurandia scandens* A. Gr. (occa-

sional at edges of valley), *Ageratum conyzoides* L. and *A. houstonianum* Mill. (rare), *Mikania scandens* L. (Wild.) (infrequent), *Eupatorium triste* DC. (few), *Baccharis scoparia* sp. (a dozen or so), *Pluchea adorata* L. (Cass.) (not infrequent), and *Bidens incisa* Ker. (frequent). All of these plants, with the possible exceptions of the *Pilea* and *Bidens*, were far less abundant than any of the four species mentioned in the preceding paragraph. Most of these fifteen plants are also smaller species, which likewise makes them less conspicuous in the vegetation of the valley. The *Duranta*, *Solanum*, and *Baccharis* are now as large as the species of *Vernonia*, but not as numerous. The climbing forms *Philibertella*, *Maurandia*, and *Mikania* of course are rather long, having already reached and spread over the tops of the largest plants near them. Many individuals of these fifteen species, as for example those growing in unusually dry situations, were dwarfed, and thus showed by their stunted form that they were not finding optimum conditions in the sterile soil and dry exposed situations afforded by the gravelly floor of the valley.

It is to be noted that, contrary to the accepted rule for invaders of new soil areas, as stated by WARMING,[4] the plants now established in the Cascade Valley are not mostly annuals or biennials. Instead they are chiefly perennials, and in fact shrubby or half-shrubby ones. Although this is true, it is to be noted also that not one arborescent form has yet been found, unless some of the young plants of *Piper* seen should prove to belong to one of the more tree-like species of this usually shrublike genus.

In this area of virgin soil there are present right through the year all of the climatic factors, such as moisture, heat, and light, that are needed for the production of a rich vegetation. This is evident from the dense forest that has developed in the adjoining valleys and even on the hills immediately overhanging the Cascade Valley itself. It was for these reasons that the writer was rather surprised, on revisiting this valley in 1919, at the slowness with which it is being recovered with vegetation. He was surprised not only at the relatively small number of new individuals, but especially at the very small number of species that had established themselves in the

[4] Oecology of plants. p. 356. 1909.

decade. It was anticipated in 1910 that certain plants which require abundant humus would not be able to settle at once on its bowlders and gravel, nor could epiphytes soon find the necessary trees to perch in. That the many mosses, ferns, and seed plants that grow all about the valley, not only in similar gravelly and stony soil along the trails, but even in the crevices of every rugged cliff and crag of the neighboring hills, should prove incapable of promptly and completely colonizing this valley was quite unexpected.

The decisive causes responsible for this slowness of revegetation have not been determined. It may be remarked in the first place that browsing by animals is a negligible factor in the development of the vegetation, since such animals are not allowed to run free in this region. Furthermore, it does not seem probable that the chemical nature of the rock can be the prime cause of this phenomenon. It is conceivable that at a later stage the soil formed by disintegration of the rock, which is an epidosite (or epidote gneiss), may determine the types of micro-organisms living in the soil and so the kinds of humus produced. The fact that a rather varied series of some thirty species of plants have been able to establish themselves in this valley shows that the soil, which is probably of fairly uniform chemical character throughout, is not especially unfavorable to plants. The distribution of the plants now growing in the valley seems rather to be related to the physical character of the soil. Plants are found growing where finer soil particles have accumulated. Probably the most important hindrance to the increase of the vegetation is instability of the soil, which, in most areas of this rather steeply sloping valley, is being constantly changed, by erosion at some points and by deposit at others.

It seems clear that in the future development of the plant covering of this valley the existing vegetation after a time will establish more fixed conditions in areas now occupied. This will give the mycorhizal fungi and soil bacteria, which cannot thrive in this sterile gravel, a sufficient amount of vegetable matter on which to feed. There will then probably be a decided acceleration both in the spread of the plant species now present, and in the introduction of new species. The writer hopes, during the coming decade, to be able to study further and to report on the progress of the revegetation of this valley.

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PECTIC MATERIAL IN ROOT HAIRS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 286

CAROLINE G. HOWE

It has been observed for some time that many soils show some acidity, that plants are able to take up much more mineral matter than can easily be extracted from soil, and also that although fertilizers are made up of soluble salts, the virgin soils are composed largely of difficultly soluble salts, and after they have been cultivated a few years, yield as full crops if not fuller than those treated with ordinary fertilizers. This has given rise to the question whether there may not be something in the structure of the root hair which enables it to change the difficultly soluble salts to such a form that they can be dissolved and taken into the plant; and since root hairs are so ephemeral, any chemical effect they may have upon the soil will not be present very long in one place.

Since many soils were found to be somewhat acid, and yet most plants cannot grow in an acid medium, two explanations were offered for this phenomenon; first, that there was an acid in the soil which, for lack of a better name, was called humus acid; and secondly, that negatively charged colloidal particles either in the plant tissues or in the soil broke up the salts and released the acids into the soil.

BAUMANN and GULLY (cited by SKENE **6**) investigated this matter with peat and mosses, and found that when put into a sodium chloride solution, these plants were able to absorb the positive ion and thus release the chlorine, which, combining with the hydrogen ion, made hydrochloric acid. SKENE (**6**) made similar tests upon sphagnum, using copper chloride solution, and found that the moss had taken up the copper, releasing the chlorine, which again formed hydrochloric acid. WIELER (**8**) also tested the higher plants, such as the needles of the pine, the leaves of the horse chestnut, American oak, and yellow lupine, and found that they were all acid, and concluded that the decaying vegetation

would explain the presence of acid in the soil. Again, ODÉN (3) found in the plants that he examined a gelatinous material of the nature of pectic acid, and MANGIN (1) found that pectose is often formed in young cells before cellulose, and that the middle lamella is calcium pectate. He (2) also found that pectose can be changed to pectic acid or pectin by gently heating in 2 per cent hydrochloric acid.

SAMPSON (5), in investigating abscission of the leaf of *Coleus*, found that there was calcium pectate in the middle lamella just before the time of abscission, but that the calcium was lacking at abscission, and discovered that this was due to the pectic acid forming so much more rapidly than the calcium was supplied that the middle lamella was broken down. Miss ROBERTS (4) also examined root hairs of a number of seedlings grown in moist air and found that they all had a layer of pectic material outside the cellulose wall, and often at the tip of the hair there was a layer of callose.

In order to determine whether this condition is general, the root hairs of twenty economic plants grown in sand and in loam were examined, and those of a few seedlings grown in Knop's solution. These seedlings were selected with the idea, first, of getting as great range as possible, and secondly, of comparing several in the same or closely related genera. These root hairs were tested microchemically for cellulose with iodine and 70 per cent sulphuric acid, which turns cellulose bright blue; for callose with resorcin blue, which causes callose to swell and to turn blue; for acidity with neutral red (and later with the Clark and Lubs indicators to determine the degree of acidity); for calcium pectate with ammonium oxalate, which unites with the calcium pectate when calcium oxalate crystals and ammonium pectate are formed; and for pectic material in general with ruthenian red. Of the special forms of pectic material chiefly found in plants, pectose is found especially in young tissues, is insoluble in water, but can be changed to pectic acid or pectin by gently heating for twenty minutes in a 2 per cent solution of hydrochloric acid. Pectin is soluble in water, and pectic acid is soluble in a 2 per cent solution of potassium hydroxide when gently heated for twenty minutes.

TABLE I

Seeds	Ammonium oxalate and calcium pectate form calcium oxalate crystals and ammonium pectate		Ruthenian red for pectic material, in general; 2 per cent potassium hydroxide for pectic acid, which is dissolved by it; 2 per cent hydrochloric acid for change of pectose: pectin soluble in water	
	Test for calcium oxalate crystals		Test for pectic material	
	Loam	Sand	Loam	Sand
Beans (Kentucky wonder)	Many crystals	Many crystals	Thick layer; pectose changed mostly to pectic acid	Pectose changed to pectin chiefly, some to pectic acid
Beans (Bush lima)	Few crystals	Few crystals	Pectose changed chiefly to pectin, some to pectic acid; thin layer	Thin layer; changed chiefly to pectic acid
Beans (Pole lima)	Many crystals, especially in older root hairs	Many crystals	Thick; pectose changed to pectic acid	Thick; pectose changed to pectic acid
Beans (Golden wax)	Very few crystals	Very few crystals	Thick layer; pectose changed to pectic acid	Same as in loam
Cabbage (Chinese)	Crystals fairly abundant	Many crystals	Pectose changed largely to pectin	Same as in loam
Cabbage (Early Jersey Wakefield)	Very few	Many crystals	Thick layer; pectose changed to pectic acid	Same as in loam
Carrot (Danver's half long)	?	?	Thick layer; pectose changed to pectic acid	Same as in loam
Corn (Yellow bantam)	Many crystals	Many crystals	Thick layer; pectose changed to pectic acid	Same as in loam
Cress (Doubled curled)	Very few	Very few, even less than in loam	Pectose changed to pectic acid	Pectose changed to pectin chiefly, some to pectic acid
Cucumber (Early fortune)	Almost no crystals	Almost no crystals	Thin layer; pectose changed mostly to pectin, some to pectic acid	Thin layer; pectose changed mostly to pectin

TABLE I—*Continued*

SEEDS	AMMONIUM OXALATE AND CALCIUM PECTATE FORM CALCIUM OXALATE CRYSTALS AND AMMONIUM PECTATE		RUTHENIAN RED FOR PECTIC MATERIAL, IN GENERAL; 2 PER CENT POTASSIUM HYDROXIDE FOR PECTIC ACID, WHICH IS DISSOLVED BY IT; 2 PER CENT HYDROCHLORIC ACID FOR CHANGE OF PECTOSE; PECTIN SOLUBLE IN WATER	
	Test for calcium oxalate crystals		Test for pectic material	
	Loam	Sand	Loam	Sand
Egg plant (Black beauty)	Many crystals	Many crystals	Thick layer; pectose changed to pectic acid	Same as in loam
Lettuce (Mignonette)	Few crystals	Many crystals, especially near tip	Pectose changed chiefly to pectic acid	Same as in loam
Parsnip (Hollow crown)	Very few crystals	Very few crystals	Pectose changed chiefly to pectic acid	Same as in loam
Peas (Telephone)	Many crystals	Many crystals	Pectose changed to pectic acid	Same as in loam
Radish (Sparkler)	Many crystals	Many crystals	Pectose changed to pectic acid	Same as in loam
Squash (Golden Hubbard)	Many crystals	Many crystals	Pectose changed chiefly to pectic acid, some to pectin	Same as in loam
Squash (Giant summer crook neck)	Number of crystals	Number of crystals	Pectose changed to pectic acid	Little pectic acid: pectose changed to pectic acid
Swiss Chard (Lucullus)	Few crystals	Few crystals	Pectose changed to pectic acid	Some pectic acid; pectose changed to pectic acid
Tomatoes (Ponderosa)	Few crystals	Few crystals	Pectose changed to pectin	Thin layer; pectose changed to pectic acid
Watermelon (Cole's early)	Many crystals	Many crystals	Some pectic acid; pectose changed to pectic acid	Same as in loam

In general, the root system was found more extensive on those seedlings grown in sand, and the root hairs were much longer.

It was also more difficult to find the young root hairs on the roots grown in sand. Pectic material was found in the outer layer of all the root hairs; some of it was in the form of calcium pectate in practically all the root hairs, much was in the form of pectose, and it was difficult to determine with certainty whether some was in the form of pectic acid. By the application of 2 per cent hydrochloric acid the pectose was changed to pectic acid except in a few instances when some was changed to pectin, and the calcium pectate was broken down to calcium chloride, allowing pectic acid to be set free. Why pectose is changed sometimes to one form and sometimes to the other is still an unsolved problem.

Callose forming an inner lamella of the wall was found in all the root hairs, being somewhat thicker at the tip, especially of the younger root hairs. The hairs grown in the two media did not differ essentially in these respects, except that the callose was somewhat thicker at the tips in loam than in sand. No cellulose was found in the root hair walls. As the root epidermal cell bulges to form a hair, the cellulose inner lamella apparently stretches to its capacity, then breaks, and no more cellulose is formed. It may be that under other conditions more cellulose would be formed.

The root hairs gave an acid reaction in all cases both in the loam and in the sand, but usually somewhat higher in the loam than in the sand. According to the P_H value, they ranged between 6.8–6.0 in the sand and in the loam, and in some cases in loam between 6.0–5.2.

The seedlings of only four species were grown in Knop's solution, and the hairs were quite numerous and symmetrical. Before the seeds were placed for germination in the Knop's solution, it was tested and found to have an acidity of 6.8–6.0. After the seedlings had grown, both the root hairs and the solution were tested for acidity. The root hairs showed about the same degree of acidity or a little less than that of the root hairs grown in the soil, while the solution was also less acid than the original, even becoming alkaline in three of the cases. These root hairs had the same structure as those grown in loam and sand, except that the callose was thicker at the tips and in two of the cases the pectose was changed to pectin.

TABLE II*

Seeds	Resorcin blue changed callose to blue		Acidity, P_H value, by use of Clark and Lubs' indicators	
	Callose		Acidity	
	Loam	Sand	Loam	Sand
Beans (Kentucky wonder)	Thick layer, especially on young hairs	Thick layer, especially on young hairs	6.0–5.2	6.0–5.2
Beans (Bush lima)	Thick layer	Thick layer	6.0–5.2	6.0–5.2
Beans (Pole lima)	Thick layer	Thick layer	6.0–5.2	6.0–5.2
Beans (Golden wax)	Fairly thick layer, especially on young hairs and at tip	Thick layer	6.0–5.2	6.8–6.0
Cabbage (Chinese)	Thin layer	Thin layer	6.8–6.0	6.8–6.0
Cabbage (Early Jersey Wakefield)	Thin layer; found on young root especially	Same as in loam	Nearer 6.0	Nearer 6.8
Carrots (Danvers)	At tip only, in young hairs, then all around	Thick in older hairs	6.0–5.2	6.0–5.2
Corn (Yellow bantam)	Thick layer, especially at tip	Same as in loam	4.6–4.4	6.0–5.2
Cress (Doubled curled)	Thick layer	Thin layer	6.0–5.2	7.6–6.8
Cucumber (Early fortune)	Thick layer	Thick layer	6.0–5.2	6.8–6.0
Egg plant (Black beauty)	Thick layer, especially at tip	Thick layer	6.0–5.2	6.0–5.2
Lettuce (Mignonette)	On younger hairs especially	Same as in loam	6.0–5.2	6.0–5.2
Parsnip (Hollow crown)	Thick layer, especially at tip	Same as in loam	6.8–6.0	7.6–6.8
Peas (Telephone)	Thick layer	Thick layer	?	6.0–5.2
Radish (Sparkler)	Some, but root hairs rather old	Layer at tip	6.8–6.0	7.6–6.8
Squash (Golden Hubbard)	Thick layer, thicker at tip	Thin layer, thicker at tip	6.0–5.2	6.8–6.0
Squash (Giant summer crookneck)	Thin layer	Fairly thick layer	6.0–5.2	6.8–6.0
Swiss Chard (Lucullus)	Thin layer, thicker at tip	Same as in loam	6.0–5.2	6.8–6.0
Tomatoes (Ponderosa)	Thick layer, especially on young hairs	Same as in loam	6.0–5.2	6.0–5.2
Watermelon (Cole's early)	Thick layer, especially on young hairs	Same as in loam	6.0–5.2	6.8–6.0

* Cellulose was not found in any of the root hairs.

From these experiments it would seem that some of the acidity of the soil is due to pectic material in the root hairs, and that this

may help change some of the difficultly soluble salts, such as tricalcium phosphate, to a soluble form that can be used by the plant.

TABLE III*

SEEDLINGS GROWN IN KNOP'S SOLUTION

SEEDS	AMMONIUM OXALATE AND CALCIUM PECTATE FORM CALCIUM OXALATE CRYSTALS AND AMMONIUM PECTATE	RUTHENIAN RED FOR PECTIC MATERIAL, IN GENERAL; 2 PER CENT HYDROCHLORIC ACID FOR CHANGE OF PECTOSE; 2 PER CENT POTASSIUM HYDROXIDE FOR PECTIC ACID	RESORCIN BLUE FOR CALLOSE
	Test for calcium oxalate crystals	Test for pectic material	Test for callose
Cabbage (Early Jersey Wakefield)	Many crystals	Thick layer all around; pectose changed mostly to pectin, some to pectic acid	Thick layer; thicker at tip
Cucumber (Early fortune)	Few crystals	Thick layer; pectose changed to pectic acid at tip, to pectin at sides	Very thick layer all round
Radish (Sparkler)	Many crystals	Pectose changed to pectic acid at tip, to pectin at sides	Thick layer, especially at tip
Muskmelon (Rockyford)	Few crystals	Pectose changed largely to pectin	Fairly thick layer

* Cellulose was not found in any of the root hairs.

TABLE IV

SEEDLINGS GROWN IN KNOP'S SOLUTION

SEEDS	ACIDITY, P_H VALUE BY USE OF CLARK AND LUBS' INDICATORS	
	Root hairs	Solution
Cabbage	6.8–6.0	7.6–7.2
Cucumber	7.2–6.8	8.4–7.6
Radish	6.8–6.0	8.4–7.6
Muskmelon	6.8–6.0	6.8–6.0

Summary

1. No cellulose was found in the root hairs of the species studied.

2. The root hairs grown in both loam and sand have a layer of pectic material on the outside, and within a layer of callose, thicker in some plants than in others, and usually a little thicker at the tips.

3. The pectic material in most of the cases at first is in the form of calcium pectate or pectose; pectic acid could not be detected with certainty. The pectic layer is somewhat thicker in loam than in sand.[1]

4. The root hairs are somewhat acid in the forms studied, and there is a tendency to be slightly more acid in loam than in sand.

5. Whether the acidity of the root hair can be ascribed to the presence of pectic material or to some other cause has not been yet determined with certainty.

Acknowledgement is due to Dr. SOPHIA H. ECKERSON for her suggestions and criticism during the progress of this study.

EAST ORANGE, N.J.

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[1] The pectose is usually changed to pectic acid by the hydrochloric acid.

DESTRUCTION OF MOSSES BY LICHENS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 287

FRANK P. MCWHORTER

(WITH PLATE XIII)

The deep-rooted conception of lichens as typical examples of symbiosis has induced workers along ecological lines to overlook the occurrence in xerarch successions of early stages which are dominated by the parasitism of lichens on mosses. This preliminary paper is intended to describe certain cases of lichen parasitism, and to emphasize the accuracy of FINK's definition of lichens: "A lichen is a fungus which lives during all or part of its life in parasitic relation with an algal host, and also sustains a relation with an organic or inorganic substratum."

The writer's attention was first called to this situation when trying to separate some *Cladonia* lichen material from a moss colony in which it was growing. The intimacy of the mixture suggested that the lichen might be to some extent parasitic on the moss. Such phenomena seem to have been noticed previously by BONNIER,[1] who shows that spores of lichens are known to germinate on moss protonemas and eventually to attack and kill them. He suggests the occurrence of such parasitism in nature on a large scale.

Moss-lichen colonies were chosen for study, in which both elements were intimately mixed, illustrating cases of dominance on the part of one or the other. It was often impossible to determine the exact species or even genus of the mosses concerned, because of the poor condition of the vegetative body and lack of reproductive organs. Mosses hampered by invading lichens seldom produce spores. Representative lichen-moss mixtures consisting of species

[1] BONNIER, GASTON, Germination des spores des Lichens sur les protonemas des Mousses et sur des Algues differents des gonidies du Lichen. Compt. Rend. Soc. Biol. Paris. 40:541–543. 1888.

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of *Dicranum*, *Bryum*, *Grimmia*, or *Fissidens* with *Cladonia*, *Physcia*, or *Amphiloma* have been collected. The following species determinations of mosses based on vegetative characters may be taken as probable: *Dicranum scoparium*, *Bryum caespiticium*, *B. argenteum*, *Grimmia apocarpa*, *G. pennsylvanica*, and *Fissidens adiantoides*. Especially important among the lichens concerned are *Cladonia cristatella*, *C. baccillaris*, *C. pyxidata*, *Physcia stellaris*, *P. obscura*, and *Amphiloma lanuginosum*.

All previous observations along this line are based on cultures and the examination of teased materials. The method employed consisted of imbedding and sectioning moss-lichen colonies, the resultant serial sections giving a veritable moving picture of the conditions in the colonies. The striking destruction of moss tissues is evident from sections 10 μ or more in thickness, but to judge the extent and nature of the haustorial action it is necessary to have sections 3 μ or less in thickness. Many kinds of fixatives were used to show to the best advantage the various tissues concerned; no one fixative gave the most satisfactory fixation for all. The fungus elements of the lichen fix well in chromoacetic; the algal elements in hot bichloride of mercury. The location of the nucleus in the algal cells, and the condition of plastids in the moss show well in aceto-formalin. The cell wall structures of all the tissues showed best in aceto-formalin. Very weak Flemming's solution gave excellent results in the young tissues of the moss. The three tissues, moss, fungus, and alga, can be sharply differentiated by a carefully balanced Flemming's triple stain. For wall studies nothing proved better than a contrasting safranin-analin-blue stain. With this stain the cell wall changes and the haustorial action may be clearly demonstrated. Slides so stained were easily photographed by suitable combinations of yellow and green filters. In addition to the section studies, a long series of cultures was run with *Amphiloma* and other lichen genera to see how readily and under what conditions they would attack a moss host.

The destructive action of lichens on moss may be seen from figs. 1 and 2. These were from 10 μ sections of intimate mixtures of *Cladonia* lichens with *Dicranum*, *Grimmia*, and other mosses,

in which the moss appears plastered over by the lichens. The apical development of the moss has been stopped. The lichen hyphae could be traced through the old moss tissue where they forced their way intercellularly.

A very constant feature of the lichen growth on mosses is the clinging of the lichen hyphae to the thickened walls of the moss. This seems to be of great significance in the eventual destruction of the moss colony. It is not the meristematic tissue that seems particularly desirable to the lichen fungus, but the thickenings of the moss walls. The case seems homologous with the destruction of wood by a polyporous fungus, where the lignified part of the wood is especially attacked, and the cellulose walls are left almost untouched. In the mosses the young walls are pure cellulose; the thickenings are of pectin. Thin sections of all the moss-lichen colonies studied showed the hyphae imbedded in the pectin. There is no evidence that the hyphae have been covered over by the forming pectin layers, but it seems obvious that the hyphae have taken their position by dissolving out the pectin. The figures of *Amphiloma* on *Grimmia* show a case of this, but *Amphiloma* is more destructive than most lichens, and in places has completely destroyed the moss. When sharply stained in safranin and analine blue, the pectinized part of the walls stains a strong red, so that penetration of the bluish stained hyphae may be plainly followed. In some colonies, even when the lichens appear to be literally plastered over the mosses, the lichen hyphae were found to be confined to the pectinized regions, and the cellulose walls to be intact; then the lichens are exerting a smothering effect carried on through a saprophytic rather than a parasitic action.

Lichen fungi sometimes become truly parasitic on their moss hosts. This is especially true of *Amphiloma*, which is shown in fig. 6. Here the lichen is an intracellular parasite. *Amphiloma* haustoria soon break down the plastids, even in old moss tissues. *Amphiloma* seldom attacks the meristematic tissues. Under some conditions *Physcia obscura* may send hyphae of non-rhizoidal nature into the meristematic moss tissues. *Physcia* also may so incorporate moss into its thallus, that the epidermis of the lichen

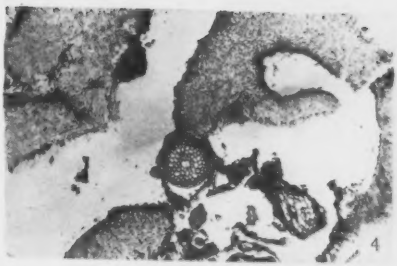
develops on the lower side of the moss leaf and the rest of the lichen on the other side, the moss becoming a veritable layer of the lichen. In such cases the moss leaf is eventually destroyed.

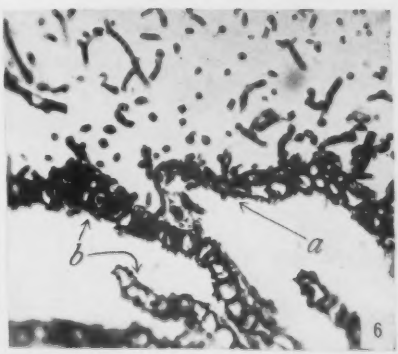
The great opportunity for the parasitizing of mosses by lichens, as they grow together in nature, cannot be over emphasized. For the most part the lichens develop on the leaves of the moss. Moss colonies in which apparently no lichens are present, when sectioned or teased out almost invariably show tiny young lichens developing in their leaves. Hundreds of lichens have been seen developing from soredial masses, but very few from spores, hence it is concluded that moss inhabiting lichens depend on soredia rather than on spores for reproduction. BONNIER'S observations on the ability of lichens to germinate on, and eventually to kill moss protonema, have already been mentioned. Since the protonemal stage is a transient one, it probably does not take place in nature to any great extent. The germination of lichens on moss leaves is the rule, so far as cases where lichens eventually plaster themselves over the mosses are concerned. The young lichen hyphae become attached from their first formation. The environmental factors control the future appearance of the colonies. From cultures and field observations it is concluded that water is the dominating factor of the control. Almost any moss colony, apparently free from lichens, when grown in semimoist conditions, but occasionally allowed to dry out, in a few weeks will produce young lichens visible to the naked eye.

If these observations are borne in mind, it is easy to see why so often the ideal lichen-moss-fern sequence is not carried out, since the sequence is broken up by lichen stages in which the lichens are more or less parasitic on the moss. If the rock surface is rough enough, visible life may be initiated by moss, and a lichen stage come in secondly. In any event a well established moss stage may be crowded out by a more or less parasitic lichen mass, which gives a secondary lichen stage succeeding the moss.

Summary

Lichens are able to destroy moss colonies. The destruction is partly due to true parasitism and partly to smothering.





McWHORTER on MOSSES AND LICHENS

The development of lichens in moss colonies makes possible the coming in of a lichen stage after the moss associations.

Great obligation is due to Professor W. J. G. LAND for suggestions in regard to the technique used, and Dr. GEO. D. FULLER for aid in the preparation of the manuscript and for reading the proof.

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EXPLANATION OF PLATE XIII

The illustrations are all photomicrographs selected from a much larger number showing similar conditions.

FIG. 1.—*Cladonia pyxidata* on moss, probably *Dicranum;* vertical section through colony showing moss plant with leaves cut to pieces and apical growth stopped by action of lichen.

FIG. 2.—*Cladonia pyxidata* on an unrecognizable moss; organization of moss leaves destroyed by action of lichen; lichen hyphae penetrated intercellularly through moss tissues.

FIG. 3.—Section of a moss-lichen mixture cut parallel to surface of colony, showing lichen (*Cladonia*) hyphae penetrated into cells of apical region of moss (*Grimmia*); hyphae indicated by arrow *a* could be traced through serial sections to lichen mass just above; *b*, moss leaf strongly attacked by hyphae.

FIG. 4.—Section from lower part of colony cut parallel to surface, showing lichen hyphae tending to fuse with pectinized walls of moss tissues.

FIG. 5.—Section through a *Amphiloma-Grimmia* mixture; *Amphiloma* has organized on moss leaf; this lichen probably destroys more moss than any other.

FIG. 6.—Portion of fig. 5 more highly magnified, showing: *a*, how lichen may completely destroy moss cells; *b*, how hyphae dissolve pectinized layer of cell walls of moss.

ANNUAL RINGS OF GROWTH IN CARBONIFEROUS WOOD

WINIFRED GOLDRING

(WITH PLATE XIV)

In a discussion of anatomical structure and climatic evolution, JEFFREY[1] emphasizes the absence of annual rings in Cordaitean wood from the Carboniferous in latitudes south of England as indicative of uniformity of climate, in contrast with the conditions in the Triassic period, in which coniferous wood with annual rings is found as far south as Arizona. His statement is as follows:

> In the Paleozoic trunks which are supplied by the geological formations of Southern Canada the organization of the wood shows great uniformity, and there are no modifications of structure which indicate any periodicity in annual conditions of growth. The truth of this statement is well illustrated by . . . . wood of a Cordaitean form from the Permo-Carboniferous of Hampton, Prince Edward Island. The presence of clear zones of periodic growth is, however, frequently found in regions of higher latitude . . . . . The next illustration shows the organization of a Carboniferous Cordaitean wood (*Mesoxylon*) from the northern part of England, and consequently of considerably higher latitude (54° N. in contrast to the 46° N., the latitute of Prince Edward Island). The annual rings in the wood from the English Carboniferous are clearly marked.
>
> For comparison with the situation revealed by the Cordaitean wood from Northern England, a trunk from the Triassic of the southwest region of the United States (Arizona) is shown . . . . . The annual rings are not so distinct in the photomicrograph as they appear on the weathered end of the actual petrified specimen. It will be clear from the information supplied in this case that as far south as Arizona in the Triassic annual rings were more or less clearly marked. A noteworthy variation in the annual temperature in that somewhat remote epoch is thus indicated.[2] This situation presents an interesting contrast to the climatic conditions which prevailed in the region of Prince Edward Island toward the end of the Paleozoic. If the situation be summarized, it is clear that in the later Paleozoic the difference between 46° N. and 54° N. means the presence in the higher latitude of annual rings and their absence in the lower one. On the other hand, in the beginning of the Mesozoic (the Triassic), even at a distance of 10° south of the latitude of Prince Edward Island, annual rings were quite clearly developed.

[1] JEFFREY, E. C., The anatomy of woody plants. 1917.

[2] This might indicate variation in moisture instead of temperature.

Recently BERRY has come into possession of part of a trunk of *Cordaites* from Bartlesville, Oklahoma, which shows annual rings of growth quite distinctly. The specimen comes from the Upper Pennsylvanian (below the Americus formation). The location is described as follows in a letter from the donor, Mr. GILBERT HART:

> As near as I can judge, the trees are confined to a rather limited belt, and are rather common there. . . . . The trees are found always below the Americus. As yet I have seen none surely in place; the nearest to the original position was in talus just below the first heavy limestone in the Admire formation. I feel sure that this is almost the true horizon.

The latitude of Bartlesville, Oklahoma, is 36°45′ N., about 10° south of the Prince Edward Island locality, practically the latitude of the Triassic forest of Arizona, where is found the coniferous wood showing more or less clearly marked annual rings. If the occurrence in Arizona argues for a "noteworthy variation in the annual temperature" in this area during the Triassic, then the annual rings in the trees of the Oklahoma forest would indicate the same for the end of the Carboniferous.

So far as known, the Oklahoma forest is the most southern occurrence of Carboniferous wood with annual rings of growth which has been noted; but such occurrences have previously been noted in wood from the Carboniferous, or earlier, in latitudes as far (or farther) south as Prince Edward Island. PENHALLOW,[3] in his discussion of North American species of *Dadoxylon*, states that, of the eighteen species now entitled to recognition, three show more or less clearly defined growth rings, while in the remaining fifteen they are obscure or obsolete. Of the species discussed in this paper one, *Cordaites pennsylvanicum* (Dawson) Penhallow, showing distinct growth rings, comes from the Carboniferous at Pittsville, Pennsylvania (41°30′ N.). Two other species, *C. Hamiltonense* Penhallow and *C. Clarkii* Dawson from the Devonian (Genesee shales) of Ontario County, New York (43°N.), show obscure growth rings. In the second species, however, they are, sometimes wanting. Both the Pennsylvanian and New York localities are much farther south than the English (54° N.) or Prince Edward Island (46° N.) areas.

[3] Trans. Roy. Soc. Can. **6**:57. 1900.

KNOWLTON,[4] in a survey of all the described species of *Cordaites* and *Dadoxylon*, describes twenty-four species as showing growth rings either distinctly or indistinctly. Of these, *Cordaites ouangondianum* Dawson from the Middle Devonian of New Brunswick and *Dadoxylon* (*Cordaites*) *annulatum* Dawson of the Middle Carboniferous of Nova Scotia must be excluded because the original descriptions were based on a complete misinterpretation of structural features (PENHALLOW, p. 56). Of the other species, nine are from the Carboniferous, the remainder from the Permian. Of the Carboniferous species, seven are from latitudes south of England, and of these four species are from latitudes as far, or practically as far south as Prince Edward Island, as follows: Nova Scotia (46° N.), three species; Niederburbach in Upper Alsace (47°45′ N.), one species showing distinct rings of growth. Most of the Permian species range in latitudes from 50°15′ N. to 51° N., but one species, with distinct growth rings, is recorded from Val d' Ajol, Department of Upper Sâone, France (47°40′ N.).

These data show that the extreme southern extension of a variable annual temperature in the Triassic period is not particularly remarkable. As far back as the Middle Devonian (Genesee) there must have been noticeable variations in climate in fairly low latitudes, in order to effect even the slight variations in wood formation noted, while in the Carboniferous the development of distinctly marked annual rings of growth indicates a pronounced seasonal variation in the climate of that period, even in far southern latitudes. This is also shown, but less markedly, in the Permian.

The specimen from the Upper Carboniferous of Bartlesville, Oklahoma, represents part of a trunk of a tree of considerable size, for in the section of trunk preserved, a radius of 5.5 inches of wood is shown with neither pith on one side nor cortex on the other. PENHALLOW gave the name *Cordaites recentium* to an undescribed species from the Permian or Permo-Carboniferous of Prince Edward Island, which Sir WILLIAM DAWSON had regarded as related, if not identical with *C. materiarium* Dawson from the Upper Carboniferous of Nova Scotia, Newfoundland, Illinois, etc. The species was not figured, but after a comparison of PENHALLOW's description

[4] Proc. U.S. Nat. Museum. **12**:601–617. 1890.

with thin sections of the Oklahoma trunk, there seems no real justification for a separation of the latter from the Prince Edward Island species, in spite of the great distance between the two localities. The original description follows:

Cordaites recentium (Dawson) Penhallow

Transverse.—Tracheids 47×53 μ broad, the walls much reduced by decay.

Radial.—Ray cells all of one kind, about equal to two tracheids; the lateral walls with round pits about one (?) per tracheid; the cells conspicuously narrower at the ends.

Bordered pits in a single row, compact, large, compressed and transversely oval or oblong, 15.6×22 μ, the orifice very variable, from oblong to round, often eccentric, but typically round and central. When distant the pits are round and smaller.

Tangential.—Rays medium, 1–2 seriate, the very broad cells 41 μ, thin-walled, round and squarish.

PENHALLOW makes no mention of the occurrence of annual rings of growth, which are very distinctly shown in the Oklahoma specimen. The rings of growth shown in the transverse section are variable in width; one has a width of 3 mm., a second 8 mm., and 6 mm. of a third are shown. The growth rings show very well on the weathered surface of the trunk; in one place the growth rings have the following successive widths: 3 mm., 3.5 mm., 7.5 mm., 3.5 mm., 3 mm., 3.5 mm., 3 mm., etc.; in another place, 4 mm., 4 mm., 4 mm., etc. On the whole, therefore, the growth rings are of about even width.

The bordered pits in a single row on the radial walls of the tracheids distinguish *C. recentium* from *C. materiarium*, in which the pits are numerous throughout the tracheids, chiefly in two, sometimes in three or four rows. The ray cells are narrowed at the ends, but not conspicuously so, and are equal to 2–6 tracheids in the Oklahoma specimen, as in *C. materiarium*, the longer cells being more frequent. The pits on the lateral walls are round, and so far as can be ascertained, one to a tracheid as described by PENHALLOW. The rays are numerous and in general very long, composed of from two to at least forty-seven cells superimposed upon each other and tapering toward each end. The rays are described as "1–2 seriate," but usually they are uniseriate. In no

place have they been found biseriate throughout. The biseriality is usually confined to the middle of the ray, although it may also occur at one or both ends; often it is confined only to the depth of one to three cells.

C. recentium resembles *Dadoxylon antiquum* Dawson (Upper Carboniferous of Nova Scotia) in the possession of bordered pits in one row, but differs from it, among other things, in the possession of practically uniseriate rays, whereas *D. antiquum* has multiseriate rays two to four cells wide. *D. prosseri* Penhallow (Permian, Chase County, Kansas) has numerous uniseriate rays (biseriate in part), but the bordered pits are smaller, and although they may occur in one row on the tracheid walls, are chiefly in two rows.

Photographic reproductions of transverse, radial, and tangential sections of the wood of this species are shown in the accompanying plate.

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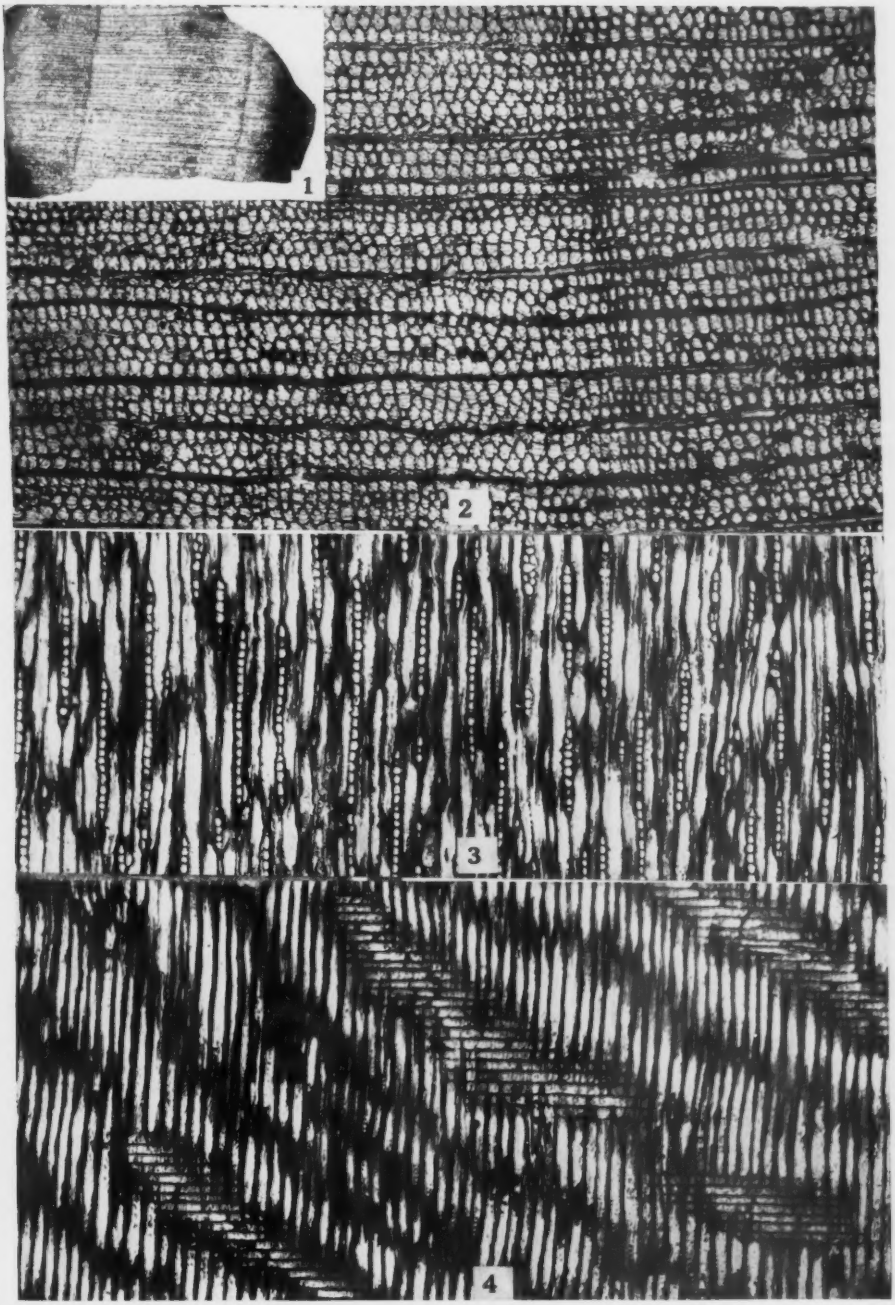
EXPLANATION OF PLATE XIV

FIG. 1.—Transverse section, ×3.
FIG. 2.—Transverse section, ×50.
FIG. 3.—Tangential section, ×50.
FIG. 4.—Radial section, ×50.



GOLDRING on CARBONIFEROUS WOOD

CURRENT LITERATURE

BOOK REVIEWS

Cytology

A book on cytology from the botanical standpoint has long been needed, and consequently botanists will welcome the vigorous, suggestive presentation of the subject by SHARP.[1] The zoological side is also presented, so that everywhere the differences and similarities in plant and animal cells are kept before the reader.

This is the first time that such a comprehensive treatment of the whole subject of cytology has been attempted by a botanist. The chapter headings, which indicate the scope of the work, are as follows: Historical sketch; Preliminary description of the cell; Protoplasm; The nucleus; The centrosome and blepharoplast; Plastids and chondriosomes; Metaplasm, polarity; Somatic mitosis and chromosome individuality; The achromatic figure, cytokinesis, and the cell wall; Other modes of nuclear division; The reduction of chromosomes; Fertilization; Apogamy, apospory, and parthenogenesis; The rôle of the cell organs in heredity; Mendelism and mutation; Sex; Linkage; Weissmanism and other theories. Any discussion or comment on details would require such an undue amount of space, that reference will only be made to the book itself.

The illustrations are numerous and exceptionally well drawn. Accompanying the descriptions of vegetative mitosis, reduction of chromosomes, and the rôle of the nucleus in heredity are numerous diagrams which will be appreciated both by students and investigators. The large number of new figures and diagrams is refreshing, and, as one turns the pages, he sees at a glance that the book owes little to shears and paste. Each subject is followed by a very full bibliography arranged alphabetically. The citations are unusually complete. The index is also commendable, with reference to figures in full faced type and with words which might be unfamiliar to some botanists or zoologists followed by an explanatory word in parenthesis, as *Eloesis* (palm), *Ectocarpus* (brown alga). *Enchenopa* (bug), etc.

SHARP's own contributions in the field of cytology, his skill as a practical technician and artist, as well as his experience in teaching the subject have fitted him for the production of this book, which will be indispensable to botanists, and should be of great value to those zoologists who feel the need of an authoritative presentation of the botanical side of cytology.—C. J. CHAMBERLAIN.

[1] SHARP, L. W., An introduction to cytology. 8vo. pp. xiii+452. New York: McGraw Hill Book Co. 1921. $4.

In Lower Florida wilds

A volume by SIMPSON,[2] described as a naturalist's observations on the geology, geography, and life of the more tropical part of the state, presents much scientific information in an attractive popular manner, well illustrated with plates from good photographs. Two chapters should be of particular interest to plant ecologists, since the one outlines the plant succession from the pine lands to the "hammocks," while the other gives a picture of the primeval forest of semitropical type. The author's opinion that the broad level evergreen forest of the "hammock" is the true climax vegetation seems well founded, while his emphasis upon the destructive and retrogressive effect of fire appears to furnish a part, at least, of the explanation why such vegetation has not dominated a larger portion of the peninsula.

The tale of the evolution of the land is attractively told, and seems as scientifically accurate as the story of the succession in the forests.—GEO. D. FULLER.

NOTES FOR STUDENTS

Organic acids and anthocyanin formation.—COMBES,[3] ROSE,[4] NICOLAS,[5] and other workers have found that anthocyanin formation is accompanied by increased oxygen fixation. MIRANDE,[6] KEEBLE[7] and ARMSTRONG, and MIEGE[8] have found that anthocyanin formation occurs regularly in tissues containing comparatively large quantities of oxidases. Later work, such as that of COMBES,[9] WILLSTÄTTER,[10] and EVEREST,[11] has shown that anthocyanins

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[2] SIMPSON, C. T., In Lower Florida wilds. 8vo. pp. xv+404. *pls. 64. maps. 2.* New York: Putnam's Sons. 1920. $3.50.

[3] COMBES, R., Les echanges gazeux des feuilles pendant la formation et la destruction des pigments anthocyaniques. Rev. Gen. Botanique **27**:177–212. 1910.

[4] ROSE, E., Étude des echanges gazeux et de la variation des sucres et des glucosides au cours de la formation des pigments anthocyaniques dans les fleurs de *Cobaea scandens*. Rev. Gen. Botanique **26**:257–270. 1914.

[5] NICOLAS, G., Contribution a l'étude des relations qui existent, dans les feuilles, entre la respiration et la presenxe de l'anthocyane. Rev. Gen. Botanique **31**:161–178. 1919.

[6] MIRANDE, M., Sur l'origine de l'anthocyanine, deduite de l'observation de quelques insectes parasites des feuilles. Compt. Rend. Acad. Sci. **145**:1300. 1907.

[7] KEEBLE, F., and ARMSTRONG, E. F., The rôle of oxidases in the formation of anthocyan pigments of plants. Jour. Genetics **2**:277–311. 1912.

[8] MIEGE, E., Recherches sur les principales espéces de *Fagopyrum*. Thesis for Doctorate. Paris. 1914.

[9] COMBES, R., Production experimentale d'une anthocyane identique a celle qui se forme dans les feuilles rouges en automne, en partant d'un compose extrait des feuilles vertes. Compt. Rend. Acad. Sci. **147**:1002–1004. 1913.

[10] WILLSTÄTTER, R., Über die Farbstoff der Bluten und Fruchte. Sitz. Ber. Akad. Wiss. 402–411. 1914.

[11] EVEREST, E., The production of anthocyanins and anthocyanidins. Proc. Roy. Soc. B. **87**:444–453. 1914.

can be produced from flavones by reduction. In the light of this work, Miss KOHLER[12] was led to believe, as NICOLAS and others previously had been, that anthocyanin formation should be correlated with organic acid accumulation, her contention being that organic substances such as carbohydrates were oxidized to organic acids, thereby reducing certain flavones and causing anthocyanin formation.

The evidence for the accumulation of organic acids during formation of red plant pigment has been more or less contradictory. WIESNER[13] and KRAUS[14] have found that acidity of the cell sap increases during autumnal reddening of leaves. ASTRUC[15] has shown that acidity descreases in petals of flowers during the reddening process. The tissues immediately beneath the red epidermis of apples were found to be less acid than tissues beneath a green epidermis in the same fruit, as determined by RIVIERE and BAILHACHE.[16] BERTHELOT and ANDRE[17] state that the amount of free acid in the plant as determined by titration of expressed juice bears no relation to the total amount of organic acid in the plant, as for the most part the acids are combined as salts of plant bases. Miss KOHLER also objects to titration of expressed juice because of the tendency of the alkali used to combine with phenolic compounds such as tannins and anthocyanins. After several unsatisfactory attempts to precipitate the phenolic compounds by the use of hide powder, zinc acetate, and analgesine (antipyrine), Miss KOHLER found that free organic acids could be quantitatively dialyzed out of the expressed juice and therefore used this method in her work. The acids were then titrated with a base and calculated as free organic acid. Combined organic acids were determined, using oven dried samples of tissue, by heating at dull red heat in a muffle. A known quantity of N/10 sulphuric acid was then added to the ash to neutralize the bases liberated by the combustion, and the acid residue titrated with alkali to find how much acid was neutralized by the ash. The figure obtained in this way was added to that of the free organic acid and the sum placed under the caption "total organic acids." Total organic acids determined in this way were found to increase in corollas of *Cobaea scandens* during the process of development from bud to mature flower, along with anthocyanin development,

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[12] KOHLER, DENISE, Étude de la variation des acides organiques an cours de la pigmentations anthocyanique. Memoir to Faculty of Science, Univ. Paris. 1921.

[13] WIESNER, J., Untersuchungen über die Herbstliche Entlaugung der Holzgewachse. Sitz. Ber. Akad. Wiss. **64**:465–510. 1871.

[14] KRAUS, C., Studien über die Herbstfarbung der Blätter und über Bildungweise der Pflanzensauren. Buchner's Repert. Pharm. **22**:273. 1873.

[15] ASTRUC, A., Recherches sur l'acidity végétale. Ann. Sci. Nat. Bot. **17**:65–109. 1903.

[16] RIVIERE, G., and BAILHACHE, G., De l'influence de la lumiere directe sur la composition chimique des fruits. Jour. Soc. Nat. Hort. France, IV. **9**:627. 1908.

[17] BERTHELOT, M., and ANDRE, G., Remarques sur la formation des acides chez les vegetaux. Compt. Rend. Acad. Sci. **32**:502. 1901.

when the flowers were allowed to remain attached to the plant. When detached there was no increase during the opening of the corolla. Leaves of *Ampelopsis tricuspidata* gathered September 17, October 1, and again on November 2, showed a progressive increase in total organic acids during the autumnal reddening. When allowed to redden detached from the plant, there was no accumulation of acids. Small plants of buckwheat, when germinated in the dark, showed a steady increase in total acids until the eighteenth day after germination. When exposed to light during this time, a red pigment developed in the hypocotyl axis, but no corresponding increase in total acids was found, either in plants attached to or detached from the parent stock. Miss KOHLER states that this fact may mean that the destruction of organic acids formed in this case is greater than their production.

It is to be regretted that Miss KOHLER has not included some similar determinations upon leaves which remain green under certain conditions and which redden under certain other conditions, in order that a comparison might be made. There is some doubt in the reviewer's mind that titration of ash, after incineration of plant tissue, gives an approximate value of the combined organic acids in the tissue before incineration. Plant tissue is a complex material. Salt combinations other than those of organic acids with inorganic bases may be altered greatly by incineration, and may leave a basic ash. Organic acids may as well be combined with organic bases within the plant and both would be lost on heating. There is even the possibility of a mixture of inorganic salts becoming more basic upon heating in a muffle. There is a tendency toward accumulation of mineral salts as the leaf ages during autumn, according to PALLADIN.[18] This accumulation might account for an increase in basicity of ash independent of color formation. In the same way migration of mineral salts into corollas and subsequent use of certain anions such as sulphates, nitrates, and phosphates in building complex compounds connected with reproduction may leave basic elements which combine in various ways and which would increase basicity of ash upon incineration. On account of the many criticisms which might be justly directed against this method of determination of combined organic acid, and on account of the insufficiency of our knowledge of complex plant compounds, it is hoped that the author of the paper will continue her studies, including some corollas which do not redden at the time of opening, and some leaves which do not redden in autumn, together with other methods for quantitatively determining the acids in question.—J. M. ARTHUR.

Vegetation of Lower California.—As the result of an expedition conducted by members of the United States Bureau of Biological Survey in 1905 and 1906,

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[18] PALLADIN, V. I., Plant physiology. p. 83. 6th. ed. transl. by LIVINGSTON, B. E., 1917.

NELSON[19] has given us a rather extensive account of the geography and resources of one of the least known regions of the continent. The larger portion of the report is occupied with an account of the exploration of the peninsula from north to south. Although 800 miles in length and from 30 to 100 miles in width, it possesses a population of little over 30,000, more than half of which is found in the extreme north on the delta plain of the Colorado River. Much of this sparcity of population is due to the essentially desert character of the peninsula as a whole. Rainfall records are very scanty, but show that there is rarely over 10 inches of annual precipitation, while over large areas from one to five years may pass with practically no rainfall. Some idea of the general aridity may be formed from the fact that throughout three-fourths of the entire length of the peninsula there are no forests whatever, only the tops of the higher mountains at the northern and southern ends being covered with trees. The only extensive forests are those contained in the northern area, where trees extend along a narrow belt 150 miles long on the higher slopes of the Juarez and San Pedro Martis Mountains, and within this area the merchantable timber does not cover more than 400 square miles. Here the more important species are *Pinus Jeffreyi*, *P. contorta*, *P. Lambertiana*, *Abies concolor*, and *Librocedrus decurrens*. Associated with them are other trees and shrubs, the same or similar species to those of southwestern California, often constituting a scattered chaparral.

The essentially desert character of the remainder of the peninsula is also shown by the inclusion of three-fourths of the entire land area within the Arid Lower Sonoran Life Zone and of more than half of the remainder within the Arid Tropical Zone. This zonal division also corresponds closely to the three main elements of the flora derived respectively from (1) the mountains and foothills of southern California, seen in the northern forests and scrub; (2) the deserts of the northwestern Sonora and southwestern United States; and (3) the lowlands and mountains of the southern Sonora on the mainland of Mexico.

Cacti appear to reach a climax in the Lower Sonoran, both in size and abundance. In his annotated list of species GOLDMAN[20] gives over 30 species belonging to 11 genera, varying in size from the smallest to such large forms as *Lemaireocereus eruca*, with huge, prostrate, caterpillar-like stems 6 or 8 ft. long, and the largest of the giants, *Pachycereus Pringlei*, more than 50 ft. high and three ft. in diameter. Many of the associated plants are the same as those of California, including species of the *Yucca*, *Agave*, *Fouqueria*, *Cercidium*, *Parkinsonia*, *Prosopis*, *Covillea*, and palms of the genera *Washingtonia* and

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[19] NELSON, E. W., Lower California and its natural resources. Mem. Nat. Acad. Sci. **16**:1–194. *pls. 1–31.* 1921.

[20] GOLDMAN, E. A., Plant records of an expedition to Lower California. Contrib. U.S. Nat. Herb. **16**:309–371. *pls. 103–133.* map. 1916.

Erythea, these last at the base of the mountains. In the southern third of the peninsula many distinctly tropical genera appear, such as *Ficus*, *Mimosa*, *Cassia*, *Albizzia*, *Jatropha*, *Haematoxylon*, *Lantana*, *Manihot*, and *Chiococca*.

Among the more remarkable endemic desert forms, two trees may be mentioned. One, belonging to the Anacardiaceae, *Pachycormus discolor*, is found in the extremely arid central section of the peninsula. Seldom 10 ft. in height, the branches often shoot out twice that distance from the trunk, while their thickness (1 ft. or more), their abrupt ending in a few short twigs covered with red flowers, "reminding one of the proboscis of an elephant holding a nosegay," give a remarkably grotesque appearance to the tree. The leaves are minute and fall off before the flowers are fully developed. The associated monotypic genus *Idria columnaris* is in the Fouqueriaceae, and appears as a tree reaching 50 ft. in height in a scattered open forest. In contrast with the preceding it has a straight columnar trunk, usually without large branches. Illustrations of these and many other interesting and unusual plants add much to the interest of both reports.—GEO. D. FULLER.

Rubus in New England.—BRAINERD and PEITERSEN,[21] recognizing that "*Rubus* is one of the most polymorphic genera in the entire plant kingdom," have presented the blackberry group of that genus as displayed in New England. The authors say that the remarkable variation in the number of species recognized in the various taxonomic works is due to too great reliance upon herbarium specimens, to failure to appreciate the variations due to environmental conditions, and to lack of appreciation of the extent of interbreeding. The present study is based upon data from material in the field, behavior in garden cultures and controlled plots, characters of the progeny of supposed natural hybrids, and behavior of progeny when artificially crossed. The result is that the authors recognize twelve valid species of New England blackberries, and a long list of hybrids.

In following up the experimental work, PEITERSEN[22] has reached the following conclusions: variations due to external factors are very marked; primordia of the prickle, glandular hair, and simple hair are present in all species; a large percentage of infertility occurs in most species, largely due to defective pollen; cross pollination is the rule in all species, all the species studied being either nearly or completely self-sterile; all the species are capable of inter-crossing under favorable conditions; duplicates of natural hybrids were produced artificially; the progeny of a number of so-called species segregated as hybrids.

The paper is a good illustration of the test of genetics applied to taxonomy. —J. M. C.

[21] BRAINERD, EZRA, and PEITERSEN, A. K., Blackberries of New England; their classification. Bull. 217, Vermont Agric. Exper. Sta. pp. 84. *pls.* 36. 1920.

[22] PEITERSEN, A. K., Blackberries of New England; genetic status of the plants. Bull. 218, Vermont Agric. Exper. Sta. pp. 34. *pls.* 19. 1921.

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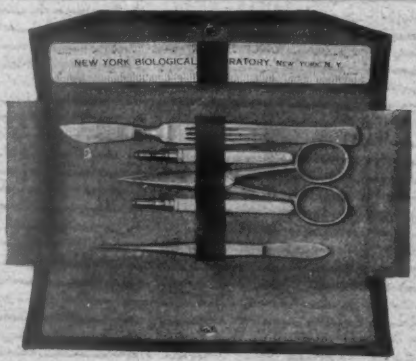

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